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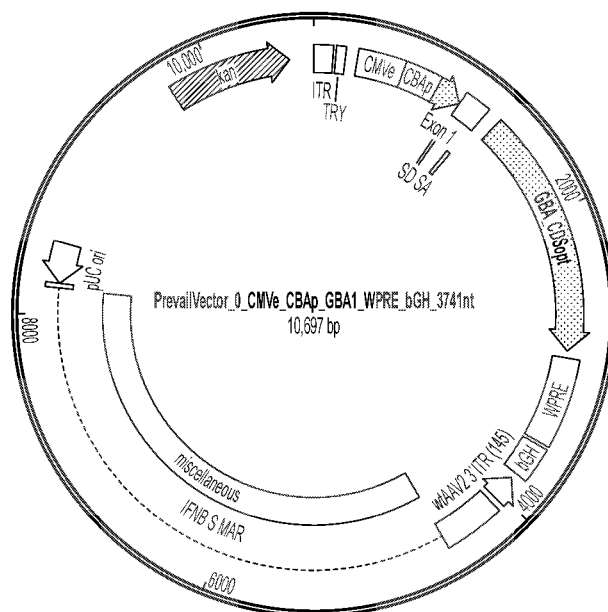


Figure 1

(57) **Abstract:** The disclosure relates, in some aspects, to compositions and methods for treatment of diseases associated with aberrant lysosomal function, for example Parkinson's disease (PD) and Gaucher disease. In some embodiments, the disclosure provides expression constructs comprising a transgene encoding beta-Glucocerebrosidase (GBA) or a portion thereof alone or in combination with one or more PD-associated genes. In some embodiments, the disclosure provides methods of Parkinson's disease by administering such expression constructs to a subject in need thereof.

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GENE THERAPIES FOR LYSOSOMAL DISORDERS

RELATED APPLICATIONS

This Application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S.

5 Provisional Application Serial Numbers 62/567,296, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, 62/567,311, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, 62/567,319, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, 62/567,301, filed October 3, 2018, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, and 62/567,310, filed
10 October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”, the entire contents of each application which are incorporated herein by reference.

BACKGROUND

Gaucher disease is a rare inborn error of glycosphingolipid metabolism due to deficiency of lysosomal acid β -glucocerebrosidase (Gcase, “GBA”). Patients suffer from non-
15 CNS symptoms and findings including hepatosplenomegaly, bone marrow insufficiency leading to pancytopenia, lung disorders and fibrosis, and bone defects. In addition, a significant number of patients suffer from neurological manifestations, including defective saccadic eye movements and gaze, seizures, cognitive deficits, developmental delay, and movement disorders including Parkinson’s disease.

20 Several therapeutics exist that address the peripheral disease and the principal clinical manifestations in hematopoietic bone marrow and viscera, including enzyme replacement therapies as described below, chaperone-like small molecule drugs that bind to defective Gcase and improve stability, and substrate reduction therapy that block the production of substrate that accumulate in Gaucher disease leading to symptoms and findings. However, other aspects of
25 Gaucher disease (particularly those affecting the skeleton and brain) appear refractory to treatment.

SUMMARY

In addition to Gaucher disease patients (who possess mutations in both chromosomal
30 alleles of *GBA1* gene), patients with mutations in only one allele of *GBA1* are at highly increased risk of Parkinson’s disease (PD). The severity of PD symptoms- which include gait difficulty, a tremor at rest, rigidity, and often depression, sleep difficulties, and cognitive decline - correlate with the degree of enzyme activity reduction. Thus, Gaucher disease patients have

the most severe course, whereas patient with a single mild mutation in *GBA1* typically have a more benign course. Mutation carriers are also at high risk of other PD-related disorders, including Lewy Body Dementia, characterized by executive dysfunction, psychosis, and a PD-like movement disorder, and multi-system atrophy, with characteristic motor and cognitive impairments. No therapies exist that alter the inexorable course of these disorders.

Deficits in enzymes such as Gcase (*e.g.*, the gene product of *GBA1* gene), as well as common variants in many genes implicated in lysosome function or trafficking of macromolecules to the lysosome (*e.g.*, Lysosomal Membrane Protein 1 (LIMP), also referred to as SCARB2), have been associated with increased PD risk. The disclosure is based, in part, on expression constructs (*e.g.*, vectors) encoding one or more PD-associated genes, for example Gcase, GBA2, prosaposin, progranulin, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, TMEM106B, or a combination of any of the foregoing (or portions thereof). In some embodiments, combinations of gene products described herein act together (*e.g.*, synergistically) to reduce one or more signs and symptoms of PD when expressed in a subject.

Accordingly, in some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a Gcase (*e.g.*, the gene product of *GBA1* gene). In some embodiments, the isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the Gcase encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 14 (*e.g.*, as set forth in NCBI Reference Sequence NP_000148.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 15. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the Gcase protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the prosaposin encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 16 (*e.g.*, as set forth in NCBI Reference Sequence NP_002769.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 17. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the prosaposin protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). In some embodiments, the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the LIMP2/SCARB2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 18 (*e.g.*, as set forth in NCBI Reference Sequence NP_005497.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 29. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the SCARB2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GBA2 protein (*e.g.*, the gene product of *GBA2* gene). In some embodiments, the isolated nucleic acid comprises a GBA2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GBA2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 30 (*e.g.*, as set forth in NCBI Reference Sequence NP_065995.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 31. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GBA2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GALC protein (*e.g.*, the gene product of *GALC* gene). In some embodiments, the isolated nucleic acid comprises a GALC-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GALC encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 33 (*e.g.*, as set forth in NCBI Reference Sequence NP_000144.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 34. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GALC protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding CTSB protein (*e.g.*, the gene product of *CTSB* gene). In some embodiments, the isolated nucleic acid comprises a CTSB-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human

cells). In some embodiments, the nucleic acid sequence encoding the CTSB encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 35 (*e.g.*, as set forth in NCBI Reference Sequence NP_001899.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 36. In some embodiments the expression construct
5 comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the CTSB protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding SMPD1 protein (*e.g.*, the gene product of *SMPD1* gene). In some embodiments, the isolated nucleic acid comprises a SMPD1-encoding sequence that has been
10 codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the SMPD1 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 37 (*e.g.*, as set forth in NCBI Reference Sequence NP_000534.3). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 38. In some embodiments the expression construct
15 comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the SMPD1 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding GCH1 protein (*e.g.*, the gene product of *GCH1* gene). In some embodiments, the isolated nucleic acid comprises a GCH1-encoding sequence that has been
20 codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the GCH1 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 45 (*e.g.*, as set forth in NCBI Reference Sequence NP_000534.3). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 46. In some embodiments the expression construct
25 comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the GCH1 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding RAB7L protein (*e.g.*, the gene product of *RAB7L* gene). In some embodiments, the isolated nucleic acid comprises a RAB7L-encoding sequence that has been
30 codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the RAB7L encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 47 (*e.g.*, as set forth in NCBI Reference Sequence NP_003920.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 48. In some embodiments the expression construct

comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the RAB7L protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding VPS35 protein (*e.g.*, the gene product of *VPS35* gene). In some
5 embodiments, the isolated nucleic acid comprises a VPS35-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the VPS35 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 49 (*e.g.*, as set forth in NCBI Reference Sequence NP_060676.2). In some embodiments, the isolated nucleic acid comprises
10 the sequence set forth in SEQ ID NO: 50. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the VPS35 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding IL-34 protein (*e.g.*, the gene product of *IL34* gene). In some
15 embodiments, the isolated nucleic acid comprises a IL-34-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the IL-34 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 55 (*e.g.*, as set forth in NCBI Reference Sequence NP_689669.2). In some embodiments, the isolated nucleic acid comprises
20 the sequence set forth in SEQ ID NO: 56. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the IL_34 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding TREM2 protein (*e.g.*, the gene product of *TREM* gene). In some
25 embodiments, the isolated nucleic acid comprises a TREM2-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the TREM2 encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 57 (*e.g.*, as set forth in NCBI Reference Sequence NP_061838.1). In some embodiments, the isolated nucleic acid comprises
30 the sequence set forth in SEQ ID NO: 58. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the TREM2 protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding TMEM106B protein (*e.g.*, the gene product of *TMEM106B* gene).

In some embodiments, the isolated nucleic acid comprises a TMEM106B-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the TMEM106B encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 63 (*e.g.*, as set forth in NCBI Reference Sequence NP_060844.2). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 64. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the TMEM106B protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding progranulin (*e.g.*, the gene product of *PGRN* gene). In some embodiments, the isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells). In some embodiments, the nucleic acid sequence encoding the progranulin (PRGN) encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 67 (*e.g.*, as set forth in NCBI Reference Sequence NP_002078.1). In some embodiments, the isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 68. In some embodiments the expression construct comprises adeno-associated virus (AAV) inverted terminal repeats (ITRs), for example AAV ITRs flanking the nucleic acid sequence encoding the prosaposin protein.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

In some embodiments, a first gene product or a second gene product is a Gcase protein, or a portion thereof. In some embodiments, a first gene product is a Gcase protein and a second gene product is selected from GBA2, prosaposin, progranulin, LIMP2, GALC, CTSB, SMPD1, GCH1, RAB7, VPS35, IL-34, TREM2, and TMEM106B.

In some embodiments, an expression construct further encodes an interfering nucleic acid (*e.g.*, shRNA, miRNA, dsRNA, *etc.*). In some embodiments, an interfering nucleic acid inhibits expression of α -Synuclein (α -Synuclein). In some embodiments, an interfering nucleic acid that targets α -Synuclein comprises a sequence set forth in any one of SEQ ID NOs: 20-25. In some embodiments, an interfering nucleic acid that targets α -Synuclein binds to (*e.g.*, hybridizes with) a sequence set forth in any one of SEQ ID NO: 20-25.

In some embodiments, an interfering nucleic acid inhibits expression of TMEM106B. In some embodiments, an interfering nucleic acid that targets TMEM106B comprises a sequence set forth in SEQ ID NO: 64 or 65. In some embodiments, an interfering nucleic acid that targets TMEM106B binds to (*e.g.*, hybridizes with) a sequence set forth in SEQ ID NO: 64 or 65.

5 In some embodiments, an expression construct further comprises one or more promoters. In some embodiments, a promoter is a chicken-beta actin (CBA) promoter, a CAG promoter, a CD68 promoter, or a JeT promoter. In some embodiments, a promoter is a RNA pol II promoter (*e.g.*, or an RNA pol III promoter (*e.g.*, U6, *etc.*)).

10 In some embodiments, an expression construct further comprises an internal ribosomal entry site (IRES). In some embodiments, an IRES is located between a first gene product and a second gene product.

In some embodiments, an expression construct further comprises a self-cleaving peptide coding sequence. In some embodiments, a self-cleaving peptide is a T2A peptide.

15 In some embodiments, an expression construct comprises two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences. In some embodiments, ITR sequences flank a first gene product and a second gene product (*e.g.*, are arranged as follows from 5'-end to 3'-end: ITR-first gene product-second gene product-ITR). In some embodiments, one of the ITR sequences of an isolated nucleic acid lacks a functional terminal resolution site (*trs*). For example, in some embodiments, one of the ITRs is a Δ ITR.

20 The disclosure relates, in some aspects, to rAAV vectors comprising an ITR having a modified "D" region (*e.g.*, a D sequence that is modified relative to wild-type AAV2 ITR, SEQ ID NO: 29). In some embodiments, the ITR having the modified D region is the 5' ITR of the rAAV vector. In some embodiments, a modified "D" region comprises an "S" sequence, for example as set forth in SEQ ID NO: 26. In some embodiments, the ITR having the modified
25 "D" region is the 3' ITR of the rAAV vector. In some embodiments, a modified "D" region comprises a 3' ITR in which the "D" region is positioned at the 3' end of the ITR (*e.g.*, on the outside or terminal end of the ITR relative to the transgene insert of the vector). In some embodiments, a modified "D" region comprises a sequence as set forth in SEQ ID NO: 26 or 27.

30 In some embodiments, an isolated nucleic acid (*e.g.*, an rAAV vector) comprises a TRY region. In some embodiments, a TRY region comprises the sequence set forth in SEQ ID NO: 28.

In some embodiments, an isolated nucleic acid described by the disclosure comprises or consists of, or encodes a peptide having, the sequence set forth in any one of SEQ ID NOs: 1-78.

In some aspects, the disclosure provides a vector comprising an isolated nucleic acid as described by the disclosure. In some embodiments, a vector is a plasmid, or a viral vector. In some embodiments, a viral vector is a recombinant AAV (rAAV) vector or a Baculovirus vector. In some embodiments, an rAAV vector is single-stranded (e.g., single-stranded DNA).

5 In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid as described by the disclosure or a vector as described by the disclosure.

In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV) comprising a capsid protein and an isolated nucleic acid or a vector as described by the disclosure.

10 In some embodiments, a capsid protein is capable of crossing the blood-brain barrier, for example an AAV9 capsid protein or an AAVrh.10 capsid protein. In some embodiments, an rAAV transduces neuronal cells and non-neuronal cells of the central nervous system (CNS).

In some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject a composition (e.g., a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

15 In some embodiments, administration comprises direct injection to the CNS of a subject. In some embodiments, direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or any combination thereof. In some
20 embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED).

In some embodiments, administration comprises peripheral injection. In some embodiments, peripheral injection is intravenous injection.

25 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof).

FIG. 2 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion thereof. The coding sequences of Gcase and LIMP2 are separated by an internal ribosomal entry site (IRES).

FIG. 3 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof) and LIMP2 (SCARB2) or a portion

thereof. Expression of the coding sequences of Gcase and LIMP2 are each driven by a separate promoter.

FIG. 4 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), LIMP2 (*SCARB2*) or a portion thereof, and an interfering RNA for α -Syn.

FIG. 5 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Prosaposin (*e.g.*, *PSAP* or a portion thereof), and an interfering RNA for α -Syn.

FIG. 6 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Prosaposin (*e.g.*, *PSAP* or a portion thereof). The coding sequences of Gcase and Prosaposin are separated by an internal ribosomal entry site (IRES).

FIG. 7 is a schematic depicting one embodiment of a vector comprising an expression construct encoding a Gcase (*e.g.*, *GBA1* or a portion thereof). In this embodiment, the vector comprises a CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAP), Exon 1, and intron (int) to constitutively express the codon optimized coding sequence of human *GBA1*. The 3' region also contains a WPRE regulatory element followed by a bGH polyA tail. Three transcriptional regulatory activation sites are included at the 5' end of the promoter region: TATA, RBS, and YY1. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (inset box) were evaluated; these have several nucleotide differences within the 20-nucleotide "D" region of wild-type AAV2 ITR. In some embodiments, an rAAV vector contains the "D" domain nucleotide sequence shown on the top line. In some embodiments, a rAAV vector comprises a mutant "D" domain (*e.g.*, an "S" domain, with the nucleotide changes shown on the bottom line).

FIG. 8 is a schematic depicting one embodiment of the vector described in FIG. 6

FIG. 9 shows representative data for delivery of an rAAV comprising a transgene encoding a Gcase (*e.g.*, *GBA1* or a portion thereof) in a CBE mouse model of Parkinson's disease. Daily IP delivery of PBS vehicle, 25 mg/kg CBE, 37.5 mg/kg CBE, or 50 mg/kg CBE (left to right) initiated at P8. Survival (top left) was checked two times a day and weight (top right) was checked daily. All groups started with $n = 8$. Behavior was assessed by total distance traveled in Open Field (bottom left) at P23 and latency to fall on Rotarod (bottom middle) at P24. Levels of the GCase substrates were analyzed in the cortex of mice in the PBS and 25 mg/kg CBE treatment groups both with (Day 3) and without (Day 1) CBE withdrawal.

Aggregate GluSph and GalSph levels (bottom right) are shown as pmol per mg wet weight of the tissue. Means are presented. Error bars are SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, nominal p-values for treatment groups by linear regression.

FIG. 10 is a schematic depicting one embodiment of a study design for maximal rAAV dose in a CBE mouse model. Briefly, rAAV was delivered by ICV injection at P3, and daily CBE treatment was initiated at P8. Behavior was assessed in the Open Field and Rotarod assays at P24-25 and substrate levels were measured at P36 and P38.

FIG. 11 shows representative data for in-life assessment of maximal rAAV dose in a CBE mouse model. At P3, mice were treated with either excipient or 8.8×10^9 vg rAAV-GBA1 via ICV delivery. Daily IP delivery of either PBS or 25 mg/kg CBE was initiated at P8. At the end of the study, half the mice were sacrificed one day after their last CBE dose at P36 (Day 1) while the remaining half went through 3 days of CBE withdrawal before sacrifice at P38 (Day3). All treatment groups (excipient + PBS $n = 8$, rAAV-GBA1 + PBS $n = 7$, excipient + CBE $n = 8$, and variant + CBE $n = 9$) were weighed daily (top left), and the weight at P36 was analyzed (top right). Behavior was assessed by total distance traveled in Open Field at P23 (bottom left) and latency to fall on Rotarod at P24 (bottom right), evaluated for each animal as the median across 3 trials. Due to lethality, $n = 7$ for the excipient + CBE group for the behavioral assays, while $n = 8$ for all other groups. Means across animals are presented. Error bars are SEM. * $p < 0.05$; *** $p < 0.001$, nominal p-values for treatment groups by linear regression in the CBE-treated animals.

FIG. 12 shows representative data for biochemical assessment of maximal rAAV dose in a CBE mouse model. The cortex of all treatment groups (excipient + PBS $n = 8$, variant + PBS $n = 7$, excipient + CBE $n = 7$, and variant + CBE $n = 9$) was used to measure GCase activity (top left), GluSph levels (top right), GluCer levels (bottom left), and vector genomes (bottom right) in the groups before (Day 1) or after (Day 3) CBE withdrawal. Biodistribution is shown as vector genomes per $1 \mu\text{g}$ of genomic DNA. Means are presented. Error bars are SEM. (* $p < 0.1$; ** $p < 0.01$; *** $p < 0.001$, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 13 shows representative data for behavioral and biochemical correlations in a CBE mouse model after administration of excipient + PBS, excipient + CBE, and variant + CBE treatment groups. Across treatment groups, performance on Rotarod was negatively correlated with GluCer accumulation (A, $p = 0.0012$ by linear regression), and GluSph accumulation was negatively correlated with increased GCase activity (B, $p = 0.0086$ by linear regression).

FIG. 14 shows representative data for biodistribution of variant in a CBE mouse model. Presence of vector genomes was assessed in the liver, spleen, kidney, and gonads for all treatment groups (excipient + PBS n = 8, variant+ PBS n = 7, excipient + CBE n = 7, and variant+ CBE n = 9). Biodistribution is shown as vector genomes per 1 µg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Means are presented. Error bars are SEM. *p<0.05; **p<0.01; ***p<0.001, nominal p-values for treatment groups by linear regression in the CBE-treated animals, with collection days and gender corrected for as covariates.

FIG. 15 shows representative data for in-life assessment of rAAV dose ranging in a CBE mouse model. Mice received excipient or one of three different doses of rAAV-GBA1 by ICV delivery at P3: 3.2e9 vg, 1.0e10vg, or 3.2e10 vg. At P8, daily IP treatment of 25 mg/kg CBE was initiated. Mice that received excipient and CBE or excipient and PBS served as controls. All treatment groups started with n = 10 (5M/5F) per group. All mice were sacrificed one day after their final CBE dose (P38-P40). All treatment groups were weighed daily, and their weight was analyzed at P36. Motor performance was assessed by latency to fall on Rotarod at P24 and latency to traverse the Tapered Beam at P30. Due to early lethality, the number of mice participating in the behavioral assays was: excipient + PBS n = 10, excipient + CBE n = 9, and 3.2e9 vg rAAV-GBA1+ CBE n = 6, 1.0e10 vg rAAV-GBA1+ CBE n = 10, 3.2e10 vg rAAV-GBA1+ CBE n = 7. Means are presented. Error bars are SEM; * p<0.05; **p<0.01 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 16 shows representative data for biochemical assessment of rAAV dose ranging in a CBE mouse model. The cortex of all treatment groups (excipient + PBS n = 10, excipient + CBE n = 9, and 3.2e9 vg rAAV-GBA1+ CBE n = 6, 1.0e10 vg rAAV-GBA1+ CBE n = 10, 3.2e10 vg rAAV-GBA1+ CBE n = 7) was used to measure GCase activity, GluSph levels, GluCer levels, and vector genomes. GCase activity is shown as ng of GCase per mg of total protein. GluSph and GluCer levels are shown as pmol per mg wet weight of the tissue. Biodistribution is shown as vector genomes per 1 µg of genomic DNA. Vector genome presence was quantified by quantitative PCR using a vector reference standard curve; genomic DNA concentration was evaluated by A260 optical density measurement. Vector genome presence was also measured in the liver (E). Means are presented. Error bars are SEM. **p<0.01; ***p<0.001 for nominal p-values by linear regression in the CBE-treated groups, with gender corrected for as a covariate.

FIG. 17 shows representative data for tapered beam analysis in maximal dose rAAV-GBA1 in a genetic mouse model. Motor performance of the treatment groups (WT + excipient, n = 5), 4L/PS-NA + excipient (n = 6), and 4L/PS-NA + rAAV-GBA1 (n = 5)) was assayed by Beam Walk 4 weeks post rAAV-GBA1 administration. The total slips and active time are shown as total over 5 trials on different beams. Speed and slips per speed are shown as the average over 5 trials on different beams. Means are presented. Error bars are SEM.

FIG. 18 shows representative data for *in vitro* expression of rAAV constructs encoding progranulin (PGRN) protein. The left panel shows a standard curve of progranulin (PGRN) ELISA assay. The bottom panel shows a dose-response of PGRN expression measured by ELISA assay in cell lysates of HEK293T cells transduced with rAAV. MOI = multiplicity of infection (vector genomes per cell).

FIG. 19 shows representative data for *in vitro* expression of rAAV constructs encoding *GBA1* in combination with Prosaposin (*PSAP*), *SCARB2*, and/or one or more inhibitory nucleic acids. Data indicate transfection of HEK293 cells with each construct resulted in overexpression of the transgenes of interest relative to mock transfected cells.

FIG. 20 is a schematic depicting an rAAV vectors comprising a “D” region located on the “outside” of the ITR (e.g., proximal to the terminus of the ITR relative to the transgene insert or expression construct) (top) and a wild-type rAAV vectors having ITRs on the “inside” of the vector (e.g., proximal to the transgene insert of the vector).

FIG. 21 a schematic depicting one embodiment of a vector comprising an expression construct encoding GBA2 or a portion thereof, and an interfering RNA for α -Syn.

FIG. 22 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof) and Galactosylceramidase (e.g., *GALC* or a portion thereof). Expression of the coding sequences of Gcase and Galactosylceramidase are separated by a T2A self-cleaving peptide sequence.

FIG. 23 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof) and Galactosylceramidase (e.g., *GALC* or a portion thereof). Expression of the coding sequences of Gcase and Galactosylceramidase are separated by a T2A self-cleaving peptide sequence.

FIG. 24 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (e.g., *GBA1* or a portion thereof), Cathepsin B (e.g., *CTSB* or a portion thereof), and an interfering RNA for α -Syn. Expression of the coding sequences of Gcase and Cathepsin B are separated by a T2A self-cleaving peptide sequence.

FIG. 25 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), Sphingomyelin phosphodiesterase 1 (*e.g.*, *SMPD1* a portion thereof, and an interfering RNA for α -Syn.

FIG. 26 is a schematic depicting one embodiment of a vector comprising an expression
5 construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Galactosylceramidase (*e.g.*, *GALC* or a portion thereof). The coding sequences of Gcase and Galactosylceramidase are separated by an internal ribosomal entry site (IRES).

FIG. 27 is a schematic depicting one embodiment of a vector comprising an expression
10 construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and Cathepsin B (*e.g.*, *CTSB* or a portion thereof). Expression of the coding sequences of Gcase and Cathepsin B are each driven by a separate promoter.

FIG. 28 is a schematic depicting one embodiment of a vector comprising an expression
15 construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), GCH1 (*e.g.*, *GCH1* or a portion thereof), and an interfering RNA for α -Syn. The coding sequences of Gcase and GCH1 are separated by an T2A self-cleaving peptide sequence

FIG. 29 is a schematic depicting one embodiment of a vector comprising an expression
construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), RAB7L1 (*e.g.*, *RAB7L1* or a portion thereof), and an interfering RNA for α -Syn. The coding sequences of Gcase and RAB7L1 are separated by an T2A self-cleaving peptide sequence.

FIG. 30 is a schematic depicting one embodiment of a vector comprising an expression
20 construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), GCH1 (*e.g.*, *GCH1* or a portion thereof), and an interfering RNA for α -Syn. Expression of the coding sequences of Gcase and GCH1 are an internal ribosomal entry site (IRES).

FIG. 31 is a schematic depicting one embodiment of a vector comprising an expression
25 construct encoding VPS35 (*e.g.*, *VPS35* or a portion thereof) and interfering RNAs for α -Syn and TMEM106B.

FIG. 32 is a schematic depicting one embodiment of a vector comprising an expression
30 construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof), IL-34 (*e.g.*, *IL34* or a portion thereof), and an interfering RNA for α -Syn. The coding sequences of Gcase and IL-34 are separated by T2A self-cleaving peptide sequence.

FIG. 33 is a schematic depicting one embodiment of a vector comprising an expression
construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and IL-34 (*e.g.*, *IL34* or a portion thereof). The coding sequences of Gcase and IL-34 are separated by an internal ribosomal entry site (IRES).

FIG. 34 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and TREM2 (*e.g.*, *TREM2* or a portion thereof). Expression of the coding sequences of Gcase and TREM2 are each driven by a separate promoter.

FIG. 35 is a schematic depicting one embodiment of a vector comprising an expression construct encoding Gcase (*e.g.*, *GBA1* or a portion thereof) and IL-34 (*e.g.*, *IL34* or a portion thereof). Expression of the coding sequences of Gcase and IL-34 are each driven by a separate promoter.

FIGs. 36A-36B show representative data for overexpression of TREM2 and GBA1 in HEK293 cells relative to control transduced cells, as measured by qPCR and ELISA. FIG. 36A shows data for overexpression of TREM2. FIG. 36B shows data for overexpression of GBA1 from the same construct.

FIG. 37 shows representative data indicating successful silencing of *SCNA in vitro* by GFP reporter assay (top) and α -Syn assay (bottom).

FIG. 38 shows representative data indicating successful silencing of *TMEM106B in vitro* by GFP reporter assay (top) and α -Syn assay (bottom).

FIG. 39 is a schematic depicting one embodiment of a vector comprising an expression construct encoding PGRN.

FIG. 40 shows data for transduction of HEK293 cells using rAAVs having ITRs with wild-type (circles) or alternative (*e.g.*, “outside”; squares) placement of the “D” sequence. The rAAVs having ITRs placed on the “outside” were able to transduce cells as efficiently as rAAVs having wild-type ITRs.

DETAILED DESCRIPTION

The disclosure is based, in part, on compositions and methods for expression of combinations of PD-associated gene products in a subject. A gene product can be a protein, a fragment (*e.g.*, portion) of a protein, an interfering nucleic acid that inhibits a PD-associated gene, *etc.* In some embodiments, a gene product is a protein or a protein fragment encoded by a PD-associated gene. In some embodiments, a gene product is an interfering nucleic acid (*e.g.*, shRNA, siRNA, miRNA, amiRNA, *etc.*) that inhibits a PD-associated gene.

A PD-associated gene refers to a gene encoding a gene product that is genetically, biochemically or functionally associated with PD. For example, individuals having mutations in the *GBA1* gene (which encodes the protein Gcase), have been observed to have an increased risk of developing PD compared to individuals that do not have a mutation in *GBA1*. In another

example, PD is associated with accumulation of protein aggregates comprising α -Synuclein (α -Syn) protein; accordingly, *SCNA* (which encodes α -Syn) is a PD-associated gene. In some embodiments, an expression cassette described herein encodes a wild-type or non-mutant form of a PD-associated gene (or coding sequence thereof). Examples of PD-associated genes are

5 listed in Table 1.

Table 1: Examples of PD-associated genes

Name	Gene	Function	NCBI Accession No.
Lysosome membrane protein 2	<i>SCARB2/LIMP2</i>	lysosomal receptor for glucosylceramidase (GBA targeting)	NP_005497.1 (Isoform 1), NP_001191184.1 (Isoform 2)
Prosaposin	<i>PSAP</i>	precursor for saposins A, B, C, and D, which localize to the lysosomal compartment and facilitate the catabolism of glycosphingolipids with short oligosaccharide groups	AAH01503.1, AAH07612.1, AAH04275.1, AAA60303.1
beta-Glucocerebrosidase	<i>GBA1</i>	cleaves the beta-glucosidic linkage of glucocerebroside	NP_001005742.1 (Isoform 1), NP_001165282.1 (Isoform 2), NP_001165283.1 (Isoform 3)
Non-lysosomal Glucosylceramidase	<i>GBA2</i>	catalyzes the conversion of glucosylceramide to free glucose and ceramide	NP_065995.1 (Isoform 1), NP_001317589.1 (Isoform 2)
Galactosylceramidase	<i>GALC</i>	removes galactose from ceramide derivatives	EAW81359.1 (Isoform CRA_a), EAW81360.1 (Isoform CRA_b), EAW81362.1 (Isoform CRA_c)
Sphingomyelin phosphodiesterase 1	<i>SMPD1</i>	converts sphingomyelin to ceramide	EAW68726.1 (Isoform CRA_a), EAW68727.1 (Isoform CRA_b), EAW68728.1 (Isoform CRA_c), EAW68729.1 (Isoform CRA_d)

Cathepsin B	<i>CTSB</i>	thiol protease believed to participate in intracellular degradation and turnover of proteins; also implicated in tumor invasion and metastasis	AAC37547.1, AAH95408.1, AAH10240.1
RAB7, member RAS oncogene family-like 1	<i>RAB7L1</i>	regulates vesicular transport	AAH02585.1
Vacuolar protein sorting-associated protein 35	<i>VPS35</i>	component of retromer cargo-selective complex	NP_060676.2
GTP cyclohydrolase 1	<i>GCH1</i>	responsible for hydrolysis of guanosine triphosphate to form 7,8-dihydroneopterin triphosphate	AAH25415.1
Interleukin 34	<i>IL34</i>	increases growth or survival of monocytes; elicits activity by binding the Colony stimulating factor 1 receptor	AAH29804.1
Triggering receptor expressed on myeloid cells 2	<i>TREM2</i>	forms a receptor signaling complex with the TYRO protein tyrosine kinase binding protein; functions in immune response and may be involved in chronic inflammation	AAF69824.1
Progranulin	<i>PGRN</i>	plays a role in development, inflammation, cell proliferation and protein homeostasis	NP_002087.1

Isolated nucleic acids and vectors

An isolated nucleic acid may be DNA or RNA. The disclosure provides, in some aspects, an isolated nucleic acids (*e.g.*, rAAV vectors) comprising an expression construct encoding one or more PD-associated genes, for example a Gcase (*e.g.*, the gene product of *GBA1* gene) or a portion thereof. Gcase, also referred to as β -glucocerebrosidase or GBA, refers to a lysosomal protein that cleaves the beta-glucosidic linkage of the chemical glucocerebroside, an intermediate in glycolipid metabolism. In humans, Gcase is encoded by the *GBA1* gene, located on chromosome 1. In some embodiments, *GBA1* encodes a peptide that is represented by NCBI Reference Sequence NCBI Reference Sequence NP_000148.2 (SEQ ID NO: 14). In some embodiments, an isolated nucleic acid comprises a Gcase-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 15.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding Prosaposin (*e.g.*, the gene product of *PSAP* gene). Prosaposin is a precursor glycoprotein for sphingolipid activator proteins (saposins) A, B, C, and D, which facilitate the catabolism of glycosphingolipids with short oligosaccharide groups. In humans, the *PSAP* gene is located on chromosome 10. In some embodiments, *PSAP* encodes a peptide that is represented by NCBI Reference Sequence NP_002769.1 (*e.g.*, SEQ ID NO: 16). In some embodiments, an isolated nucleic acid comprises a prosaposin-encoding sequence that has been codon optimized (*e.g.*, codon optimized for expression in mammalian cells, for example human cells), such as the sequence set forth in SEQ ID NO: 17.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding LIMP2/SCARB2 (*e.g.*, the gene product of *SCARB2* gene). SCARB2 refers to a membrane protein that regulates lysosomal and endosomal transport within a cell. In humans, *SCARB2* gene is located on chromosome 4. In some embodiments, the *SCARB2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_005497.1 (SEQ ID NO: 18). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 19. In some embodiments the isolated nucleic acid comprises a SCARB2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GBA2 protein (*e.g.*, the gene product of *GBA2* gene). GBA2 protein refers to non-lysosomal glucosylceramidase. In humans, *GBA2* gene is located on chromosome 9. In some embodiments, the *GBA2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_065995.1 (SEQ ID NO: 30). In some embodiments, an isolated nucleic acid

comprises the sequence set forth in SEQ ID NO: 31. In some embodiments the isolated nucleic acid comprises a GBA2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GALC protein (*e.g.*, the gene product of *GALC* gene). GALC protein refers to galactosylceramidase (or galactocerebrosidase), which is an enzyme that hydrolyzes galactose ester bonds of galactocerebroside, galactosylsphingosine, lactosylceramide, and monogalactosyldiglyceride. In humans, *GALC* gene is located on chromosome 14. In some embodiments, the *GALC* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000144.2 (SEQ ID NO: 33). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 34. In some embodiments the isolated nucleic acid comprises a GALC-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding CTSB protein (*e.g.*, the gene product of *CTSB* gene). CTSB protein refers to cathepsin B, which is a lysosomal cysteine protease that plays an important role in intracellular proteolysis. In humans, *CTSB* gene is located on chromosome 8. In some embodiments, the *CTSB* gene encodes a peptide that is represented by NCBI Reference Sequence NP_001899.1 (SEQ ID NO: 35). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 36. In some embodiments the isolated nucleic acid comprises a CTSB-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding SMPD1 protein (*e.g.*, the gene product of *SMPD1* gene). SMPD1 protein refers to sphingomyelin phosphodiesterase 1, which is a hydrolase enzyme that is involved in sphingolipid metabolism. In humans, *SMPD1* gene is located on chromosome 11. In some embodiments, the *SMPD1* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000534.3 (SEQ ID NO: 37). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 38. In some embodiments the isolated nucleic acid comprises a SMPD1-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding GCH1 protein (*e.g.*, the gene product of *GCH1* gene). GCH1 protein refers to GTP cyclohydrolase I, which is a hydrolase enzyme that is part of the folate and bipterin biosynthesis pathways. In humans, *GCH1* gene is located on chromosome 14. In some embodiments, the *GCH1* gene encodes a peptide that is represented by NCBI Reference Sequence NP_000152.1 (SEQ ID NO: 45). In some embodiments, an isolated nucleic acid

comprises the sequence set forth in SEQ ID NO: 46. In some embodiments the isolated nucleic acid comprises a GCH1-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding RAB7L protein (*e.g.*, the gene product of *RAB7L* gene). RAB7L protein refers to RAB7, member RAS oncogene family-like 1, which is a GTP binding protein. In humans, *RAB7L* gene is located on chromosome 1. In some embodiments, the *RAB7L* gene encodes a peptide that is represented by NCBI Reference Sequence NP_003920.1 (SEQ ID NO: 47). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 48. In some embodiments the isolated nucleic acid comprises a RAB7L-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding VPS35 protein (*e.g.*, the gene product of *VPS35* gene). VPS35 protein refers to vacuolar protein sorting-associated protein 35, which is part of a protein complex involved in retrograde transport of proteins from endosomes to the trans-Golgi network. In humans, *VPS35* gene is located on chromosome 16. In some embodiments, the *VPS35* gene encodes a peptide that is represented by NCBI Reference Sequence NP_060676.2 (SEQ ID NO: 49). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 50. In some embodiments the isolated nucleic acid comprises a VPS35-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding IL-34 protein (*e.g.*, the gene product of *IL34* gene). IL-34 protein refers to interleukin 34, which is a cytokine that increases growth and survival of monocytes. In humans, *IL34* gene is located on chromosome 16. In some embodiments, the *IL34* gene encodes a peptide that is represented by NCBI Reference Sequence NP_689669.2 (SEQ ID NO: 55). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 56. In some embodiments the isolated nucleic acid comprises a IL-34-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding TREM2 protein (*e.g.*, the gene product of *TREM2* gene). TREM2 protein refers to triggering receptor expressed on myeloid cells 2, which is an immunoglobulin superfamily receptor found on myeloid cells. In humans, *TREM2* gene is located on chromosome 6. In some embodiments, the *TREM2* gene encodes a peptide that is represented by NCBI Reference Sequence NP_061838.1 (SEQ ID NO: 57). In some embodiments, the

isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 58. In some embodiments an isolated nucleic acid comprises a TREM2-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding TMEM106B protein (*e.g.*, the gene product of *TMEM106B* gene).

5 TMEM106B protein refers to transmembrane protein 106B, which is a protein involved in dendrite morphogenesis and regulation of lysosomal trafficking. In humans, *TMEM106B* gene is located on chromosome 7. In some embodiments, the *TMEM106B* gene encodes a peptide that is represented by NCBI Reference Sequence NP_060844.2 (SEQ ID NO: 62). In some
10 embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 63. In some embodiments the isolated nucleic acid comprises a TMEM106B-encoding sequence that has been codon optimized.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding progranulin protein (*e.g.*, the gene product of *PRGN* gene). PGRN protein refers to progranulin, which is a protein involved in development, inflammation, cell
15 proliferation and protein homeostasis. In humans, *PGRN* gene is located on chromosome 17. In some embodiments, the *PGRN* gene encodes a peptide that is represented by NCBI Reference Sequence NP_002078.1 (SEQ ID NO: 66). In some embodiments, an isolated nucleic acid comprises the sequence set forth in SEQ ID NO: 67. In some embodiments the isolated nucleic acid comprises a PGRN-encoding sequence that has been codon optimized.

20 In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

In some embodiments, a gene product is encoded by a coding portion (*e.g.*, a cDNA) of a
25 naturally occurring gene. In some embodiments, a first gene product is a protein (or a fragment thereof) encoded by the *GBA1* gene. In some embodiments, a gene product is a protein (or a fragment thereof) encoded by another gene listed in Table 1, for example the *SCARB2/LIMP2* gene or the *PSAP* gene. However, the skilled artisan recognizes that the order of expression of a first gene product (*e.g.*, Gcase) and a second gene product (*e.g.*, LIMP2, *etc.*) can generally be
30 reversed (*e.g.*, LIMP2 is the first gene product and Gcase is the second gene product). In some embodiments, a gene product is a fragment (*e.g.*, portion) of a gene listed in Table 1. A protein fragment may comprise about 50%, about 60%, about 70%, about 80% about 90% or about 99% of a protein encoded by the genes listed in Table 1. In some embodiments, a protein fragment

comprises between 50% and 99.9% (*e.g.*, any value between 50% and 99.9%) of a protein encoded by a gene listed in Table 1.

In some embodiments, an expression construct is monocistronic (*e.g.*, the expression construct encodes a single fusion protein comprising a first gene product and a second gene product). In some embodiments, an expression construct is polycistronic (*e.g.*, the expression construct encodes two distinct gene products, for example two different proteins or protein fragments).

A polycistronic expression vector may comprise a one or more (*e.g.*, 1, 2, 3, 4, 5, or more) promoters. Any suitable promoter can be used, for example, a constitutive promoter, an inducible promoter, an endogenous promoter, a tissue-specific promoter (*e.g.*, a CNS-specific promoter), *etc.* In some embodiments, a promoter is a chicken beta-actin promoter (CBA promoter), *etc.* In some embodiments, a promoter is a chicken beta-actin promoter (CBA promoter), a CAG promoter (for example as described by Alexopoulou et al. (2008) *BMC Cell Biol.* 9:2; doi: 10.1186/1471-2121-9-2), a CD68 promoter, or a JeT promoter (for example as described by Tornøe et al. (2002) *Gene* 297(1-2):21-32). In some embodiments, a promoter is operably-linked to a nucleic acid sequence encoding a first gene product, a second gene product, or a first gene product and a second gene product. In some embodiments, an expression cassette comprises one or more additional regulatory sequences, including but not limited to transcription factor binding sequences, intron splice sites, poly(A) addition sites, enhancer sequences, repressor binding sites, or any combination of the foregoing.

In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding an internal ribosomal entry site (IRES). Examples of IRES sites are described, for example, by Mokrejs et al. (2006) *Nucleic Acids Res.* 34(Database issue):D125-30. In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding a self-cleaving peptide. Examples of self-cleaving peptides include but are not limited to T2A, P2A, E2A, F2A, BmCPV 2A, and BmIFV 2A, and those described by Liu et al. (2017) *Sci Rep.* 7: 2193. In some embodiments, the self-cleaving peptide is a T2A peptide.

Pathologically, disorders such as PD and Gaucher disease are associated with accumulation of protein aggregates composed largely of α -Synuclein (α -Syn) protein. Accordingly, in some embodiments, isolated nucleic acids described herein comprise an inhibitory nucleic acid that reduces or prevents expression of α -Syn protein. A sequence encoding an inhibitory nucleic acid may be placed in an untranslated region (*e.g.*, intron, 5'UTR, 3'UTR, *etc.*) of the expression vector.

In some embodiments, an inhibitory nucleic acid is positioned in an intron of an expression construct, for example in an intron upstream of the sequence encoding a first gene product. An inhibitory nucleic acid can be a double stranded RNA (dsRNA), siRNA, micro RNA (miRNA), artificial miRNA (amiRNA), or an RNA aptamer. Generally, an inhibitory nucleic acid binds to (*e.g.*, hybridizes with) between about 6 and about 30 (*e.g.*, any integer between 6 and 30, inclusive) contiguous nucleotides of a target RNA (*e.g.*, mRNA). In some embodiments, the inhibitory nucleic acid molecule is an miRNA or an amiRNA, for example an miRNA that targets *SNCA* (the gene encoding α -Syn protein) or *TMEM106B* (*e.g.*, the gene encoding TMEM106B protein). In some embodiments, the miRNA does not comprise any mismatches with the region of *SNCA* mRNA to which it hybridizes (*e.g.*, the miRNA is “perfected”). In some embodiments, the inhibitory nucleic acid is an shRNA (*e.g.*, an shRNA targeting *SNCA* or *TMEM106B*). In some embodiments, an inhibitory nucleic acid is an artificial miRNA (amiRNA) that includes a miR-155 scaffold and a *SNCA* or *TMEM106B* targeting sequence.

An isolated nucleic acid as described herein may exist on its own, or as part of a vector. Generally, a vector can be a plasmid, cosmid, phagemid, bacterial artificial chromosome (BAC), or a viral vector (*e.g.*, adenoviral vector, adeno-associated virus (AAV) vector, retroviral vector, baculoviral vector, *etc.*). In some embodiments, the vector is a plasmid (*e.g.*, a plasmid comprising an isolated nucleic acid as described herein). In some embodiments, an rAAV vector is single-stranded (*e.g.*, single-stranded DNA). In some embodiments, the vector is a recombinant AAV (rAAV) vector. In some embodiments, a vector is a Baculovirus vector (*e.g.*, an *Autographa californica* nuclear polyhedrosis (AcNPV) vector).

Typically an rAAV vector (*e.g.*, rAAV genome) comprises a transgene (*e.g.*, an expression construct comprising one or more of each of the following: promoter, intron, enhancer sequence, protein coding sequence, inhibitory RNA coding sequence, polyA tail sequence, *etc.*) flanked by two AAV inverted terminal repeat (ITR) sequences. In some embodiments the transgene of an rAAV vector comprises an isolated nucleic acid as described by the disclosure. In some embodiments, each of the two ITR sequences of an rAAV vector is a full-length ITR (*e.g.*, approximately 145 bp in length, and containing functional *Rep* binding site (RBS) and terminal resolution site (*trs*)). In some embodiments, one of the ITRs of an rAAV vector is truncated (*e.g.*, shortened or not full-length). In some embodiments, a truncated ITR lacks a functional terminal resolution site (*trs*) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a truncated ITR is a Δ ITR, for example as described by McCarty et al. (2003) *Gene Ther.* 10(26):2112-8.

Aspects of the disclosure relate to isolated nucleic acids (*e.g.*, rAAV vectors) comprising an ITR having one or more modifications (*e.g.*, nucleic acid additions, deletions, substitutions, *etc.*) relative to a wild-type AAV ITR, for example relative to wild-type AAV2 ITR (*e.g.*, SEQ ID NO: 29). The structure of wild-type AAV2 ITR is shown in FIG. 20. Generally, a wild-type ITR comprises a 125 nucleotide region that self-anneals to form a palindromic double-stranded T-shaped, hairpin structure consisting of two cross arms (formed by sequences referred to as B/B' and C/C', respectively), a longer stem region (formed by sequences A/A'), and a single-stranded terminal region referred to as the "D" region (FIG. 20). Generally, the "D" region of an ITR is positioned between the stem region formed by the A/A' sequences and the insert containing the transgene of the rAAV vector (*e.g.*, positioned on the "inside" of the ITR relative to the terminus of the ITR or proximal to the transgene insert or expression construct of the rAAV vector). In some embodiments, a "D" region comprises the sequence set forth in SEQ ID NO: 27. The "D" region has been observed to play an important role in encapsidation of rAAV vectors by capsid proteins, for example as disclosed by Ling et al. (2015) *J Mol Genet Med* 9(3).

The disclosure is based, in part, on the surprising discovery that rAAV vectors comprising a "D" region located on the "outside" of the ITR (*e.g.*, proximal to the terminus of the ITR relative to the transgene insert or expression construct) are efficiently encapsidated by AAV capsid proteins than rAAV vectors having ITRs with unmodified (*e.g.*, wild-type) ITRs. In some embodiments, rAAV vectors having a modified "D" sequence (*e.g.*, a "D" sequence in the "outside" position) have reduced toxicity relative to rAAV vectors having wild-type ITR sequences.

In some embodiments, a modified "D" sequence comprises at least one nucleotide substitution relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). A modified "D" sequence may have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 nucleotide substitutions relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence comprises at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 nucleic acid substitutions relative to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence is between about 10% and about 99% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%) identical to a wild-type "D" sequence (*e.g.*, SEQ ID NO: 27). In some embodiments, a modified "D" sequence comprises the sequence set forth in SEQ ID NO: 26, also referred to as an "S" sequence as described in Wang et al. (1995) *J Mol Biol* 250(5):573-80.

An isolated nucleic acid or rAAV vector as described by the disclosure may further comprise a "TRY" sequence, for example as set forth in SEQ ID NO: 28 or as described by

Francois, et al. 2005. The Cellular TATA Binding Protein Is Required for Rep-Dependent Replication of a Minimal Adeno-Associated Virus Type 2 p5 Element. J Virol. In some embodiments, a TRY sequence is positioned between an ITR (e.g. a 5' ITR) and an expression construct (e.g. a transgene-encoding insert) of an isolated nucleic acid or rAAV vector.

5 In some aspects, the disclosure relates to Baculovirus vectors comprising an isolated nucleic acid or rAAV vector as described by the disclosure. In some embodiments, the Baculovirus vector is an *Autographa californica* nuclear polyhedrosis (AcNPV) vector, for example as described by Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43 and Smith et al. (2009) *Mol Ther* 17(11):1888-1896.

10 In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or vector as described herein. A host cell can be a prokaryotic cell or a eukaryotic cell. For example, a host cell can be a mammalian cell, bacterial cell, yeast cell, insect cell, etc. In some embodiments, a host cell is a mammalian cell, for example a HEK293T cell. In some embodiments, a host cell is a bacterial cell, for example an *E. coli* cell.

15 rAAVs

In some aspects, the disclosure relates to recombinant AAVs (rAAVs) comprising a transgene that encodes a nucleic acid as described herein (e.g., an rAAV vector as described herein). The term "rAAVs" generally refers to viral particles comprising an rAAV vector
20 encapsidated by one or more AAV capsid proteins. An rAAV described by the disclosure may comprise a capsid protein having a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAV10. In some embodiments, an rAAV comprises a capsid protein from a non-human host, for example a rhesus AAV capsid protein such as AAVrh.10, AAVrh.39, etc. In some embodiments, an rAAV described by the disclosure
25 comprises a capsid protein that is a variant of a wild-type capsid protein, such as a capsid protein variant that includes at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 (e.g., 15, 20, 25, 50, 100, etc.) amino acid substitutions (e.g., mutations) relative to the wild-type AAV capsid protein from which it is derived.

30 In some embodiments, rAAVs described by the disclosure readily spread through the CNS, particularly when introduced into the CSF space or directly into the brain parenchyma. Accordingly, in some embodiments, rAAVs described by the disclosure comprise a capsid protein that is capable of crossing the blood-brain barrier (BBB). For example, in some embodiments, an rAAV comprises a capsid protein having an AAV9 or AAVrh.10 serotype.

Production of rAAVs is described, for example, by Samulski et al. (1989) *J Virol.* 63(9):3822-8 and Wright (2009) *Hum Gene Ther.* 20(7): 698–706.

In some embodiments, an rAAV as described by the disclosure (*e.g.*, comprising a recombinant rAAV genome encapsidated by AAV capsid proteins to form an rAAV capsid particle) is produced in a Baculovirus vector expression system (BEVS). Production of rAAVs using BEVS are described, for example by Urabe et al. (2002) *Hum Gene Ther* 13(16):1935-43, Smith et al. (2009) *Mol Ther* 17(11):1888-1896, U.S. Patent No. 8,945,918, U.S. Patent No. 9,879,282, and International PCT Publication WO 2017/184879. However, an rAAV can be produced using any suitable method (*e.g.*, using recombinant rep and cap genes).

Pharmaceutical Compositions

In some aspects, the disclosure provides pharmaceutical compositions comprising an isolated nucleic acid or rAAV as described herein and a pharmaceutically acceptable carrier. As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *e.g.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function. Additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

Compositions (*e.g.*, pharmaceutical compositions) provided herein can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration via blood and/or lymph supply, and/or direct administration to an affected site.

In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration). In certain embodiments, the compound or pharmaceutical composition described herein is suitable for topical administration to the eye of a subject.

Methods

The disclosure is based, in part, on compositions for expression of combinations of PD-associated gene products in a subject that act together (*e.g.*, synergistically) to treat Parkinson's disease. As used herein "treat" or "treating" refers to (a) preventing or delaying onset of Parkinson's disease; (b) reducing severity of Parkinson's disease; (c) reducing or preventing development of symptoms characteristic of Parkinson's disease; (d) and/or preventing worsening of symptoms characteristic of Parkinson's disease. Symptoms of Parkinson's disease include, for example, motor dysfunction (*e.g.*, shaking, rigidity, slowness of movement, difficulty with walking), cognitive dysfunction (*e.g.*, dementia, depression, anxiety), emotional and behavioral dysfunction.

Accordingly, in some aspects, the disclosure provides a method for treating a subject having or suspected of having Parkinson's disease, the method comprising administering to the subject a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure.

In some embodiments, a composition is administered directly to the CNS of the subject, for example by direct injection into the brain and/or spinal cord of the subject. Examples of CNS-direct administration modalities include but are not limited to intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing. In some embodiments, direct injection into the CNS of a subject results in transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the midbrain, striatum and/or cerebral cortex of the subject. In some embodiments, direct injection into the CNS results in transgene expression (*e.g.*, expression of the first gene product, second gene product, and if applicable, third gene product) in the spinal cord and/or CSF of the subject.

In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). Convection enhanced delivery is a therapeutic strategy that involves surgical exposure of the brain and placement of a small-diameter catheter directly into a target area of the brain, followed by infusion of a therapeutic agent (*e.g.*, a composition or rAAV as

described herein) directly to the brain of the subject. CED is described, for example by Debinski et al. (2009) *Expert Rev Neurother.* 9(10):1519-27.

In some embodiments, a composition is administered peripherally to a subject, for example by peripheral injection. Examples of peripheral injection include subcutaneous
5 injection, intravenous injection, intra-arterial injection, intraperitoneal injection, or any combination of the foregoing. In some embodiments, the peripheral injection is intra-arterial injection, for example injection into the carotid artery of a subject.

In some embodiments, a composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure is administered both peripherally and
10 directly to the CNS of a subject. For example, in some embodiments, a subject is administered a composition by intra-arterial injection (*e.g.*, injection into the carotid artery) and by intraparenchymal injection (*e.g.*, intraparenchymal injection by CED). In some embodiments, the direct injection to the CNS and the peripheral injection are simultaneous (*e.g.*, happen at the same time). In some embodiments, the direct injection occurs prior (*e.g.*, between 1 minute and
15 1 week, or more before) to the peripheral injection. In some embodiments, the direct injection occurs after (*e.g.*, between 1 minute and 1 week, or more after) the peripheral injection.

The amount of composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure administered to a subject will vary depending on the administration method. For example, in some embodiments, a rAAV as described herein
20 is administered to a subject at a titer between about 10^9 Genome copies (GC)/kg and about 10^{14} GC/kg (*e.g.*, about 10^9 GC/kg, about 10^{10} GC/kg, about 10^{11} GC/kg, about 10^{12} GC/kg, about 10^{12} GC/kg, or about 10^{14} GC/kg). In some embodiments, a subject is administered a high titer (*e.g.*, $>10^{12}$ Genome Copies GC/kg of an rAAV) by injection to the CSF space, or by intraparenchymal injection.

A composition (*e.g.*, a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure can be administered to a subject once or multiple times
25 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, or more) times. In some embodiments, a composition is administered to a subject continuously (*e.g.*, chronically), for example via an infusion pump.

EXAMPLES

Example 1: rAAV vectors

AAV vectors are generated using cells, such as HEK293 cells for triple-plasmid transfection. The ITR sequences flank an expression construct comprising a promoter/enhancer element for each transgene of interest, a 3' polyA signal, and posttranslational signals such as the WPRE element. Multiple gene products can be expressed simultaneously such as *GBA1* and *LIMP2* and/or Prosaposin, by fusion of the protein sequences; or using a 2A peptide linker, such as T2A or P2A, which leads 2 peptide fragments with added amino acids due to prevention of the creation of a peptide bond; or using an IRES element; or by expression with 2 separate expression cassettes. The presence of a short intronic sequence that is efficiently spliced, upstream of the expressed gene, can improve expression levels. shRNAs and other regulatory RNAs can potentially be included within these sequences. Examples of expression constructs described by the disclosure are shown in FIGs. 1-8 and 21-35, and in Table 2 below.

Table 2

Name	Promoter 1	shRNA	CDS1	PolyA 1	Bicistronic element	Promoter 2	CDS2	PolyA2	Length between ITRs
CMVe_CBAP_GBA1_WPRE_bGH	CBA		GBA1	WPRE-bGH					3741
LT1s_JetLong_mRNAi aSYn_SCARB2-T2A-GBA1_bGH	JetLong	aSyn	SCARB2	bGH	T2A		GBA1		4215
LI1_JetLong_SCARB2-IRES-GBA1_bGH	JetLong		SCARB2	bGH	IRES		GBA1		4399
FP1_JetLong_GBA1_bGH_JetLong_SCARB2_SV40L	JetLong		GBA1	bGH		JetLong	SCARB2	SV40L	4464
PrevailVector_LT2s_JetLong_mRNAi aSYn_P SAP-T2A-GBA1_bGH_4353nt	JetLong	aSyn	PSAP	bGH	T2A	-	GBA1	-	4353
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1_SyntheticpolyA_4337nt	JetLong	-	PSAP	Synthetic pA	IRES	-	GBA1	-	4337

PrevailVector_10s_JetLong_mRNAiaSyn_GBA2_WPRE_bGH_4308nt	JetLong	aSyn	GBA2	WPRE_bGH	-	-	-	-	4308
PrevailVector_FT4_JetLong_GBA1_T2A_GALC_SyntheticpolyA_4373nt	JetLong	-	GBA1	Synthetic pA	T2A	-	GALC	-	4373
PrevailVector_LT4_JetLong_GALC_T2A_GBA1_SyntheticpolyA_4373nt	JetLong	-	GALC	Synthetic pA	T2A	-	GBA1	-	4373
PrevailVector_LT5s_JetLong_mRNAiaSyn_CTSB-T2A-GBA1_WPRE_bGH_4392nt	JetLong	aSyn	CTSB	WPRE_bGH	T2A	-	GBA1	-	4392
PrevailVector_FT11t_JetLong_mRNAiaSyn_GBA1_T2S_SMPD1_SyntheticpolyA_4477nt	JetLong	aSyn	GBA1	Synthetic pA	T2A	-	SMPD1	-	4477
PrevailVector_LI4_JetLong_GALC_IRES_GBA1_SyntheticpolyA_4820nt	JetLong	-	GALC	Synthetic pA	IRES	-	GBA1	-	4820
PrevailVector_FP5_JetLong_GBA1_bGH_JetLong_CTSB_SV40L_4108nt	JetLong	-	GBA1	bGH	-	JetLong	CTSB	SV40L	4108
PrevailVector_FT6s_JetLong_mRNAiaSyn_GBA1-T2A-GCH1_WPRE_bGH_4125nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	GCH1	-	4125
PrevailVector_LT7s_JetLong_mRNAiaSyn_RAB7L1-T2A-GBA1_WPRE_bGH_3984nt	JetLong	aSyn	RAB7L1	WPRE_bGH	T2A	-	GBA1	-	3984
PrevailVector_FI6s_JetLong_mRNAiaSYn_G	JetLong	aSyn	GBA1	bGH	IRES	-	GCH1	-	3978

BA1-IRES-GCH1_bGH_3978nt									
PrevailVector_9st_JetLong_mRNAiaSyn_mRNAiTMEM106B_VPS35_WPRE_bGH_4182nt	JetLong	aSyn & TMEM106B	VPS35	WPRE_bGH	-	-	-	-	4182
PrevailVector_FT12s_JetLong_mRNAiaSyn_GBA1-T2A-IL34_WPRE_bGH_4104nt	JetLong	aSyn	GBA1	WPRE_bGH	T2A	-	IL34	-	4104
PrevailVector_FI12s_JetLong_mRNAiaSYn_GBA1-IRES-IL34_bGH_3957nt	JetLong	aSyn	GBA1	bGH	IRES	-	IL34	-	3957
PrevailVector_FP8_JetLong_GBA1_bGH_CD68_TREM2_SV40L_4253nt	JetLong	-	GBA1	bGH	-	CD68	TREM2	SV40L	4253
PrevailVector_FP12_CMV_CBA_GBA1_bGH_JetLong_IL34_SV40L_4503nt	CBA		GBA1	bGH		JetLong	IL34	SV40L	4503

Example 2: Cell based assays of viral transduction into GBA-deficient cells

Cells deficient in *GBA1* are obtained, for example as fibroblasts from GD patients, monocytes, or hES cells, or patient-derived induced pluripotent stem cells (iPSCs). These cells
5 accumulate substrates such as glucosylceramide and glucosylsphingosine (GlcCer and GlcSph). Treatment of wild-type or mutant cultured cell lines with Gcase inhibitors, such as CBE, is also be used to obtain GBA deficient cells.

Using such cell models, lysosomal defects are quantified in terms of accumulation of protein aggregates, such as of α -Synuclein with an antibody for this protein or phospho- α Syn,
10 followed by imaging using fluorescent microscopy. Imaging for lysosomal abnormalities by ICC for protein markers such as LAMP1, LAMP2, LIMP1, LIMP2, or using dyes such as LysoTracker, or by uptake through the endocytic compartment of fluorescent dextran or other markers is also performed. Imaging for autophagy marker accumulation due to defective fusion

with the lysosome, such as for LC3, can also be performed. Western blotting and/or ELISA is used to quantify abnormal accumulation of these markers. Also, the accumulation of glycolipid substrates and products of GBA1 is measured using standard approaches.

Therapeutic endpoints (*e.g.*, reduction of PD-associated pathology) are measured in the context of expression of transduction of the AAV vectors, to confirm and quantify activity and function. Gcase can also be quantified using protein ELISA measures, or by standard Gcase activity assays.

Example 3: In vivo assays using mutant mice

This example describes *in vivo* assays of AAV vectors using mutant mice. *In vivo* studies of AAV vectors as above in mutant mice are performed using assays described, for example, by Liou *et al.* (2006) *J. Biol. Chem.* 281(7): 4242–4253, Sun *et al.* (2005) *J. Lipid Res.* 46:2102–2113, and Farfel-Becker *et al.* (2011) *Dis. Model Mech.* 4(6):746–752.

The intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example at an injection volume between 5–10 μ L. Intraparenchymal delivery by convection enhanced delivery is performed.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α -Synuclein monomers, protofibrils or fibrils.

Example 4: Chemical models of disease

This example describes *in vivo* assays of AAV vectors using a chemically-induced mouse model of Gaucher disease (*e.g.*, the CBE mouse model). *In vivo* studies of these AAV vectors are performed in a chemically-induced mouse model of Gaucher disease, for example as described by Vardi *et al.* (2016) *J Pathol.* 239(4):496-509.

Intrathecal or intraventricular delivery of vehicle control and AAV vectors (*e.g.*, at a dose of 2×10^{11} vg/mouse) are performed using concentrated AAV stocks, for example with injection volume between 5–10 μ L. Intraparenchymal delivery by convection enhanced delivery is performed. Peripheral delivery is achieved by tail vein injection.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of

Gcase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, lysosomal dysfunction, and accumulation of α -Synuclein monomers, protofibrils or fibrils.

Example 5: Clinical trials in PD, LBD, Gaucher disease patients

5 In some embodiments, patients having certain forms of Gaucher disease (*e.g.*, GD1) have an increased risk of developing Parkinson's disease (PD) or Lewy body dementia (LBD). This Example describes clinical trials to assess the safety and efficacy of rAAVs as described by the disclosure, in patients having Gaucher disease, PD and/or LBD.

Clinical trials of such vectors for treatment of Gaucher disease, PD and/or LBD are
10 performed using a study design similar to that described in Grabowski et al. (1995) *Ann. Intern. Med.* 122(1):33-39.

Example 6: Treatment of peripheral disease

In some embodiments, patients having certain forms of Gaucher disease exhibit
15 symptoms of peripheral neuropathy, for example as described in Biegstraaten *et al.* (2010) *Brain* 133(10):2909–2919.

This example describes *in vivo* assays of AAV vectors as described herein for treatment of peripheral neuropathy associated with Gaucher disease (*e.g.*, Type 1 Gaucher disease). Briefly, Type 1 Gaucher disease patients identified as having signs or symptoms of peripheral
20 neuropathy are administered a rAAV as described by the disclosure. In some embodiments, the peripheral neuropathic signs and symptoms of the subject are monitored, for example using methods described in Biegstraaten *et al.*, after administration of the rAAV.

Levels of transduced gene products as described by the disclosure present in patients (*e.g.*, in serum of a patient, in peripheral tissue (*e.g.*, liver tissue, spleen tissue, *etc.*)) of a patient
25 are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 7: Treatment of CNS forms

This example describes *in vivo* assays of rAAVs as described herein for treatment of
30 CNS forms of Gaucher disease. Briefly, Gaucher disease patients identified as having a CNS form of Gaucher disease (*e.g.*, Type 2 or Type 3 Gaucher disease) are administered a rAAV as described by the disclosure. Levels of transduced gene products as described by the disclosure present in the CNS of patients (*e.g.*, in serum of the CNS of a patient, in cerebrospinal fluid

(CSF) of a patient, or in CNS tissue of a patient) are assayed, for example by Western blot analysis, enzymatic functional assays, or imaging studies.

Example 8: Gene therapy of Parkinson's Disease in subjects having mutations in GBA1

5 This example describes administration of a recombinant adeno-associated virus (rAAV) encoding *GBA1* to a subject having Parkinson's disease characterized by a mutation in *GBA1* gene.

The rAAV-GBA1 vector insert contains the CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVe), CBA promoter (CBAP), Exon 1, and intron (int) to
10 constitutively express the codon optimized coding sequence (CDS) of human GBA1 (maroon). The 3' region also contains a Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) posttranscriptional regulatory element followed by a bovine Growth Hormone polyA signal (bGH polyA) tail. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5' ITR sequence (FIG. 7, inset box, bottom sequence) were
15 evaluated; these variants have several nucleotide differences within the 20-nucleotide "D" region of the ITR, which is believed to impact the efficiency of packaging and expression. The rAAV-GBA1 vector product contains the "D" domain nucleotide sequence shown in FIG. 7 (inset box, top sequence). A variant vector harbors a mutant "D" domain (termed an "S" domain herein, with the nucleotide changes shown by shading), performed similarly in preclinical
20 studies. The backbone contains the gene to confer resistance to kanamycin as well as a stuffer sequence to prevent reverse packaging. A schematic depicting a rAAV-GBA1 vector is shown in FIG. 8. The rAAV-GBA1 vector is packaged into an rAAV using AAV9 serotype capsid proteins.

rAAV-GBA1 is administered to a subject as a single dose via a fluoroscopy guided sub-
25 occipital injection into the cisterna magna (intracisternal magna; ICM). One embodiment of a rAAV-GBA1 dosing regimen study is as follows:

A single dose of rAAV-GBA1 is administered to patients (N=12) at one of two dose levels (3e13 vg (low dose); 1e14 vg (high dose), *etc.*) which are determined based on the results of nonclinical pharmacology and toxicology studies.

30 Initial studies were conducted in a chemical mouse model involving daily delivery of conduritol-b-epoxide (CBE), an inhibitor of GCase to assess the efficacy and safety of the rAAV-GBA1 vector and a rAAV-GBA1 S-variant construct (as described further below). Additionally, initial studies were performed in a genetic mouse model, which carries a homozygous *GBA1* mutation and is partially deficient in saposins (4L/PS-NA). Additional

dose-ranging studies in mice and nonhuman primates (NHPs) are conducted to further evaluate vector safety and efficacy.

Two slightly different versions of the 5' inverted terminal repeat (ITR) in the AAV backbone were tested to assess manufacturability and transgene expression (FIG. 7). The 20 bp "D" domain within the 145 bp 5' ITR is thought to be necessary for optimal viral vector production, but mutations within the "D" domain have also been reported to increase transgene expression in some cases. Thus, in addition to the viral vector rAAV-GBA1, which harbors an intact "D" domain, a second vector form with a mutant D domain (termed an "S" domain herein) was also evaluated. Both rAAV-GBA1 and the variant express the same transgene. While both vectors produced virus that was efficacious in vivo as detailed below, rAAV-GBA1, which contains a wild-type "D" domain, was selected for further development.

To establish the CBE model of GCase deficiency, juvenile mice were dosed with CBE, a specific inhibitor of GCase. Mice were given CBE by IP injection daily, starting at postnatal day 8 (P8). Three different CBE doses (25 mg/kg, 37.5 mg/kg, 50 mg/kg) and PBS were tested to establish a model that exhibits a behavioral phenotype (FIG. 9). Higher doses of CBE led to lethality in a dose-dependent manner. All mice treated with 50 mg/kg CBE died by P23, and 5 of the 8 mice treated with 37.5 mg/kg CBE died by P27. There was no lethality in mice treated with 25 mg/kg CBE. Whereas CBE-injected mice showed no general motor deficits in the open field assay (traveling the same distance and at the same velocity as mice given PBS), CBE-treated mice exhibited a motor coordination and balance deficit as measured by the rotarod assay.

Mice surviving to the end of the study were sacrificed on the day after their last CBE dose (P27, "Day 1") or after three days of CBE withdrawal (P29, "Day 3"). Lipid analysis was performed on the cortex of mice given 25 mg/kg CBE to evaluate the accumulation of GCase substrates in both the Day 1 and Day 3 cohorts. GluSph and GalSph levels (measured in aggregate in this example) were significantly accumulated in the CBE-treated mice compared to PBS-treated controls, consistent with GCase insufficiency.

Based on the study described above, the 25 mg/kg CBE dose was selected since it produced behavioral deficits without impacting survival. To achieve widespread GBA1 distribution throughout the brain and transgene expression during CBE treatment, rAAV-GBA1 or excipient was delivered by intracerebroventricular (ICV) injection at postnatal day 3 (P3) followed by daily IP CBE or PBS treatment initiated at P8 (FIG. 10).

CBE-treated mice that received rAAV-GBA1 performed statistically significantly better on the rotarod than those that received excipient (FIG. 11). Mice in the variant treatment group

did not differ from excipient treated mice in terms of other behavioral measures, such as the total distance traveled during testing (FIG. 11).

At the completion of the in-life study, half of the mice were sacrificed the day after the last CBE dose (P36, “Day 1”) or after three days of CBE withdrawal (P38, “Day 3”) for biochemical analysis (FIG. 12). Using a fluorometric enzyme assay performed in biological triplicate, GCase activity was assessed in the cortex. GCase activity was increased in mice that were treated with rAAV-GBA1, while CBE treatment reduced GCase activity. Additionally, mice that received both CBE and rAAV-GBA1 had GCase activity levels that were similar to the PBS-treated group, indicating that delivery of rAAV-GBA1 is able to overcome the inhibition of GCase activity induced by CBE treatment. Lipid analysis was performed on the motor cortex of the mice to examine levels of the substrates GluCer and GluSph. Both lipids accumulated in the brains of mice given CBE, and rAAV-GBA1 treatment significantly reduced substrate accumulation.

Lipid levels were negatively correlated with both GCase activity and performance on the Rotarod across treatment groups. The increased GCase activity after rAAV-GBA1 administration was associated with substrate reduction and enhanced motor function (FIG. 13). As shown in FIG. 14, preliminary biodistribution was assessed by vector genome presence, as measured by qPCR (with >100 vector genomes per 1 µg genomic DNA defined as positive). Mice that received rAAV-GBA1, both with and without CBE, were positive for rAAV-GBA1 vector genomes in the cortex, indicating that ICV delivery results in rAAV-GBA1 delivery to the cortex. Additionally, vector genomes were detected in the liver, few in spleen, and none in the heart, kidney or gonads. For all measures, there was no statistically significant difference between the Day 1 and Day 3 groups.

A larger study in the CBE model further explored efficacious doses of rAAV-GBA1 in the CBE model. Using the 25 mg/kg CBE dose model, excipient or rAAV-GBA1 was delivered via ICV at P3, and daily IP PBS or CBE treatment initiated at P8. Given the similarity between the groups with and without CBE withdrawal observed in the previous studies, all mice were sacrificed one day after the final CBE dose (P38-40). The effect of three different rAAV-GBA1 doses was assessed, resulting in the following five groups, with 10 mice (5M/5F) per group:

Excipient ICV + PBS IP

Excipient ICV + 25 mg/kg CBE IP

3.2e9 vg (2.13e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

3.2e10 vg (2.13e11 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP.

The highest dose of rAAV-GBA1 rescued the CBE treatment-related failure to gain weight at P37. Additionally, this dose resulted in a statistically significant increase in performance on the rotarod and tapered beam compared to the Excipient + CBE treated group (FIG. 15). Lethality was observed in several groups, including both excipient-treated and rAAV-GBA1-treated groups (Excipient + PBS: 0; Excipient + 25 mg/kg CBE: 1; 3.2e9 vg rAAV-GBA1+ 25 mg/kg CBE: 4; 1.0e10 vg rAAV-GBA1+ 25 mg/kg CBE: 0; 3.2e10 vg rAAV-GBA1+ 25 mg/kg CBE: 3).

At the completion of the in-life study, mice were sacrificed for biochemical analysis (FIG. 16). GCCase activity in the cortex was assessed in biological triplicates by a fluorometric assay. CBE-treated mice showed reduced GCCase activity whereas mice that received a high rAAV-GBA1 dose showed a statistically significant increase in GCCase activity compared to CBE treatment. CBE-treated mice also had accumulation of GluCer and GluSph, both of which were rescued by administering a high dose of rAAV-GBA1.

In addition to the established chemical CBE model, rAAV-GBA1 is also evaluated in the 4L/PS-NA genetic model, which is homozygous for the V394L GD mutation in Gba1 and is also partially deficient in saposins, which affect GCCase localization and activity. These mice exhibit motor strength, coordination, and balance deficits, as evidenced by their performance in the beam walk, rotarod, and wire hang assays. Typically the lifespan of these mice is less than 22 weeks. In an initial study, 3 μ l of maximal titer virus was delivered by ICV at P23, with a final dose of 2.4e10 vg (6.0e10 vg/g brain). With 6 mice per group, the treatment groups were:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 2.4e10 vg (6.0e10 vg/g brain) rAAV-GBA1 ICV

Motor performance by the beam walk test was assessed 4 weeks post- rAAV-GBA1 delivery. The group of mutant mice that received rAAV-GBA1 showed a trend towards fewer total slips and fewer slips per speed when compared to mutant mice treated with excipient, restoring motor function to near WT levels (FIG. 17). Since the motor phenotypes become more severe as these mice age, their performance on this and other behavioral tests is assessed at later time points. At the completion of the in-life study, lipid levels, GCCase activity, and biodistribution are assessed in these mice.

Additional lower doses of rAAV-GBA1 are currently being tested using the CBE model, corresponding to 0.03x, 0.1x, and 1x the proposed phase 1 high clinical dose. Each group includes 10 mice (5M/5F) per group:

Excipient ICV

Excipient ICV + 25 mg/kg CBE IP

3.2e8 vg (2.13e9 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e9 vg (6.67e9 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP

1.0e10 vg (6.67e10 vg/g brain) rAAV-GBA1 ICV + 25 mg/kg CBE IP.

In addition to motor phenotypes, lipid levels and GCase activity are assessed in the cortex. Time course of treatments and analyses are also performed.

A larger dose ranging study was initiated to evaluate efficacy and safety data. 10 4L/PS-NA mice (5M/5F per group) were injected with 10 µl of rAAV-GBA1. Using an allometric brain weight calculation, the doses correlate to 0.15x, 1.5x, 4.4x, and 14.5x the proposed phase 1 high clinical dose. The injection groups consist of:

WT + Excipient ICV

4L/PS-NA + Excipient ICV

4L/PS-NA + 4.3e9 vg (1.1e10 vg/g brain) rAAV-GBA1 ICV

4L/PS-NA + 4.3e10 vg (1.1e11 vg/g brain) rAAV-GBA1 ICV

4L/PS-NA + 1.3e11 vg (3.2e11 vg/g brain) rAAV-GBA1 ICV

4L/PS-NA + 4.3e11 vg (1.1e12 vg/g brain) rAAV-GBA1 ICV.

A summary of nonclinical studies in the CBE model are shown in Table 3 below.

Table 3: Summary of Results in CBE Mouse Model

Test Material	Study Number	Dose Cohort	Behavioral Changes			Lipids	Enzyme	BD	
			Rotarod	Tapered Beam	Open Field			Brain	Liver
rAAV-GBA1	PRV-2018-005 Dose-ranging	3.2e9 vg (2.13e10 vg/g brain)	NS	NS	NS	NS	NS	+	-

	rAAV-GBA1 in CBE Model	1.10e10 vg (6.67e10 vg/g brain)	T	NS	NS	T/S	NS	+	+
		2.3e10vg (2.13e11 vg/g brain)	S	S	NS	S	S	+	+
Variant	PRV-2018-005 Dose-ranging Variant in CBE Model	8.8e9 vg (5.9e10 vg/g brain)	S	N/A	NS	S	S	+	+

Note that positive biodistribution is defined as >100 vg/1 µg genomic DNA.

Abbreviations: BD = biodistribution; NS = nonsignificant; T = trend; S = significant; N/A = not applicable; + = positive; - = negative.

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Example 9: In vitro analysis of rAAV vectors

rAAV constructs were tested *in vitro* and *in vivo*. FIG. 18 shows representative data for in vitro expression of rAAV constructs encoding progranulin (PGRN) protein. The left panel shows a standard curve of progranulin (PGRN) ELISA assay. The bottom panel shows a dose-response of PGRN expression measured by ELISA assay in cell lysates of HEK293T cells transduced with rAAV. MOI = multiplicity of infection (vector genomes per cell).

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A pilot study was performed to assess *in vitro* activity of rAAV vectors encoding Prosaposin (*PSAP*) and *SCARB2*, alone or in combination with *GBA1* and/or one or more inhibitory RNAs. One construct encoding PSAP and progranulin (PGRN) was also tested. Vectors tested include those shown in Table 4. “Opt” refers to a nucleic acid sequence codon optimized for expression in mammalian cells (*e.g.*, human cells). FIG. 19 shows representative data indicating that transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

15

A pilot study was performed to assess *in vitro* activity of rAAV vectors encoding TREM2, alone or in combination with one or more inhibitory RNAs. Vectors tested include those shown in Table 4. “Opt” refers to a nucleic acid sequence codon optimized for expression in mammalian cells (*e.g.*, human cells). FIGs. 36A-36B show representative data indicating that

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transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

Table 4

ID	Promoter	Inhibitory RNA	Promoter	Transgene
I00015	JL_intronic	SCNA	JetLong	Opt-PSAP_GBA1
I00039	-	-	JetLong	Opt-PSAP-GRN
I00046	-	-	CBA	Opt-PSAP
I00014	JetLong	SCNA	JetLong	Opt-SCARB2_GBA1
I00040			JL, CD68	opt-GBA1, TREM2

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Example 10: Testing of SCNA and TMEM106B shRNA constructs

HEK293 cells

Human embryonic kidney 293 cell line (HEK293) were used in this study (#85120602, Sigma-Aldrich). HEK293 cells were maintained in culture media (D-MEM [#11995065, Thermo Fisher Scientific] supplemented with 10% fetal bovine serum [FBS] [#10082147, Thermo Fisher Scientific]) containing 100 units/ml penicillin and 100 µg/ml streptomycin (#15140122, Thermo Fisher Scientific).

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Plasmid transfection

Plasmid transfection was performed using Lipofectamine 2000 transfection reagent (#11668019, Thermo Fisher Scientific) according to the manufacture's instruction. Briefly, HEK293 cells (#12022001, Sigma-Aldrich) were plated at the density of 3×10^5 cells/ml in culture media without antibiotics. On the following day, the plasmid and Lipofectamine 2000 reagent were combined in Opti-MEM solution (#31985062, Thermo Fisher Scientific). After 5 minutes, the mixtures were added into the HEK293 culture. After 72 hours, the cells were harvested for RNA or protein extraction, or subjected to the imaging analyses. For imaging analyses, the plates were pre-coated with 0.01% poly-L-Lysine solution (P8920, Sigma-Aldrich) before the plating of cells.

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Gene expression analysis by quantitative real-time PCR (qRT-PCR)

Relative gene expression levels were determined by quantitative real-time PCR (qRT-PCR) using Power SYBR Green Cells-to-CT Kit (#4402955, Thermo Fisher Scientific) according to the manufacturer's instruction. The candidate plasmids were transiently transfected into HEK293 cells plated on 48-well plates (7.5×10^4 cells/well) using Lipofectamine 2000 transfection reagent (0.5 μ g plasmid and 1.5 μ l reagent in 50 μ l Opti-MEM solution). After 72 hours, RNA was extracted from the cells and used for reverse transcription to synthesize cDNA according to the manufacturer's instruction. For quantitative PCR analysis, 2~5 μ l of cDNA products were amplified in duplicates using gene specific primer pairs (250 nM final concentration) with Power SYBR Green PCR Master Mix (#4367659, Thermo Fisher Scientific). The primer sequences for *SNCA*, *TMEM106B*, and *GAPDH* genes were: 5'- AAG AGG GTG TTC TCT ATG TAG GC -3' (SEQ ID NO: 71), 5'- GCT CCT CCA ACA TTT GTC ACT T -3' (SEQ ID NO: 72) for *SNCA*, 5'-ACA CAG TAC CTA CCG TTA TAG CA-3' (SEQ ID NO: 73), 5'-TGT TGT CAC AGT AAC TTG CAT CA-3' (SEQ ID NO: 74) for *TMEM106B*, and 5'- CTG GGC TAC ACT GAG CAC C -3' (SEQ ID NO: 75), 5'- AAG TGG TCG TTG AGG GCA ATG -3' (SEQ ID NO: 76) for *GAPDH*. Quantitative PCR was performed in a QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific). Expression levels were normalized by the housekeeping gene *GAPDH* and calculated using the comparative CT method.

Fluorescence Imaging Analysis

EGFP reporter plasmids, which contain 3'-UTR of human *SNCA* gene at downstream of EGFP coding region, were used for the validation of *SNCA* and *TMEM106B* knockdown plasmids. EGFP reporter plasmids and candidate knockdown plasmids were simultaneously transfected into HEK293 cells plated on poly-L-Lysine coated 96-well plates (3.0×10^4 cells/well) using Lipofectamine 2000 transfection reagent (0.04 μ g reporter plasmid, 0.06 μ g knockdown plasmid and 0.3 μ l reagent in 10 μ l Opti-MEM solution). After 72 hours, the fluorescent intensities of EGFP signal were measured at excitation 488 nm/emission 512 nm using Varioskan LUX multimode reader (Thermo Fisher Scientific). Cells were fixed with 4% PFA at RT for 10 minutes, and incubated with D-PBS containing 40 μ g/ml 7-aminoactinomycin D (7-AAD) for 30 min at RT. After washing with D-PBS, the fluorescent intensities of 7-AAD signal were measured at excitation 546 nm/emission 647 nm using Varioskan reader to quantify cell number. Normalized EGFP signal per 7-AAD signal levels were compared with the control knockdown samples.

Enzyme-linked Immunosorbent Assay (ELISA)

α -Synuclein reporter plasmids, which contain 3'-UTR of human *SNCA* gene or *TMEM106B* gene downstream of *SNCA* coding region, were used for the validation of knockdown plasmids at the protein level. Levels of α -synuclein protein were determined by ELISA (#KHB0061, Thermo Fisher Scientific) using the lysates extracted from HEK293 cells. The candidate plasmids were transiently transfected into HEK293 cells plated on 48-well plates (7.5×10^4 cells/well) using Lipofectamine 2000 transfection reagent (0.1 μ g reporter plasmid, 0.15 μ g knockdown plasmid and 0.75 μ l reagent in 25 μ l Opti-MEM solution). After 72 hours, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (#89900, Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (#P8340, Sigma-Aldrich), and sonicated for a few seconds. After incubation on ice for 30 min, the lysates were centrifuged at $20,000 \times g$ at $4^\circ C$ for 15 min, and the supernatant was collected. Protein levels were quantified. Plates were read in a Varioskan plate reader at 450 nm, and concentrations were calculated using SoftMax Pro 5 software. Measured protein concentrations were normalized to total protein concentration determined with a bicinchoninic acid assay (#23225, Thermo Fisher Scientific).

FIG. 37 and Table 5 show representative data indicating successful silencing of *SCNA* *in vitro* by GFP reporter assay (top) and α -Syn assay (bottom). FIG. 38 and Table 6 show representative data indicating successful silencing of *TMEM106B* *in vitro* by GFP reporter assay (top) and α -Syn assay (bottom).

Table 5

ID	Promoter	Knockdown	Promoter	Overexpress
I00007	CMV_intronic	SNCA_mi	CMV	opt-GBA1
I00008	H1	SNCA_sh	CMV	opt-GBA1
I00009	H1	SNCA_Pubsh4	CMV	opt-GBA1
I00014	JL_intronic	SNCA_mi	JetLong	opt-SCARB2_GBA
I00015	JL_intronic	SNCA_mi	JetLong	opt-PSAP_GBA
I00016	JL_intronic	SNCA_mi	JetLong	opt-CTSB_GBA

I00019	JL_intronic	SNCA_TMEN_mi	JetLong	opt-VPS35
I00023	JL_intronic	SNCA_mi	JetLong	opt-GBA1_IL34
I00024	JL_intronic	SNCA_mi	JetLong	opt-GBA2
I00028	intronic	SNCA_Broadsh	CMV	opt-GBA1
I00029	intronic	SNCA_Pubsh4	CMV	opt-GBA1

Table 6

ID	Promoter	Knockdown	Promoter	Overexpress
I00010	H1	TMEN_Pubsh	CMV	opt-GRN
I00011	JL_intronic	TMEN_mi	JetLong	opt-GBA1_GRN
I00012	H1	TMEN_sh	CMV	opt-GRN
I00019	JL_intronic	SNCA_TMEN_mi	JetLong	opt-VPS35

Example 11: ITR “D” sequence placement and cell transduction

5 The effect of placement of ITR “D” sequence on cell transduction of rAAV vectors was investigated. HEK293 cells were transduced with Gcase-encoding rAAVs having 1) wild-type ITRs (e.g., “D” sequences proximal to the transgene insert and distal to the terminus of the ITR) or 2) ITRs with the “D” sequence located on the “outside” of the vector (e.g., “D” sequence located proximal to the terminus of the ITR and distal to the transgene insert), as shown in FIG.

10 20. Surprisingly, data indicate that rAAVs having the “D” sequence located in the “outside” position retain the ability to be packaged and transduce cells efficiently (FIG. 40).

Example 12: In vitro testing of Progranulin rAAVs

15 FIG. 39 is a schematic depicting one embodiment of a vector comprising an expression construct encoding PGRN. Progranulin is overexpressed in the CNS of rodents deficient in GRN, either heterozygous or homozygous for GRN deletion, by injection of an rAAV vector

encoding PGRN (*e.g.*, codon-optimized PGRN), either by intraparenchymal or intrathecal injection such as into the cisterna magna.

Mice are injected at 2 months or 6 months of age, and aged to 6 months or 12 months and analyzed for one or more of the following: expression level of GRN at the RNA and protein levels, behavioral assays (*e.g.*, improved movement), survival assays (*e.g.*, improved survival), microglia and inflammatory markers, gliosis, neuronal loss, Lipofuscinosis, and/or Lysosomal marker accumulation rescue, such as LAMP1. Assays on PGRN-deficient mice are described, for example by Arrant et al. (2017) *Brain* 140: 1477-1465; Arrant et al. (2018) *J. Neuroscience* 38(9):2341–2358; and Amado et al. (2018) doi:<https://doi.org/10.1101/30869>; the entire contents of which are incorporated herein by reference.

EQUIVALENTS

This Application incorporates by reference the contents of the following documents in their entirety: the International PCT Application referred to by Attorney Docket Number P1094.70002WO00, filed October 3 2018; International PCT Application referred to by Attorney Docket Number P1094.70004WO00, filed October 3, 2018; Provisional Application Serial Numbers 62/567,296, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; 62/567,311, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; 62/567,319, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; 62/567,301, filed October 3, 2018, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; 62/567,310, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; 62/567,303, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”; and 62/567,305, filed October 3, 2017, entitled “GENE THERAPIES FOR LYSOSOMAL DISORDERS”.

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention.

Accordingly, the foregoing description and drawings are by way of example only.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be

within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings
5 of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as
10 specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

15 The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements
20 may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A
25 (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); *etc.*

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also
30 including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of

exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); *etc.*

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Use of ordinal terms such as “first,” “second,” “third,” *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

SEQUENCES

In some embodiments, an expression cassette encoding one or more gene products (*e.g.*, a first, second and/or third gene product) comprises or consists of (or encodes a peptide having) a sequence set forth in any one of SEQ ID NOs: 1-78. In some embodiments, a gene product is encoded by a portion (*e.g.*, fragment) of any one of SEQ ID NOs: 1-78.

By way of clarification and for avoidance of doubt, as used herein and except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additions, components, integers or steps.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising
 - (i) an expression construct comprising a chicken-beta actin (CBA) promoter, a CMV enhancer, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a Bovine Growth Hormone polyA signal tail, and a transgene encoding a Progranulin (PGRN) protein, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 68; and
 - (ii) two adeno-associated virus (AAV) inverted terminal repeats (ITR) sequences flanking the expression construct, wherein the first ITR sequence is a 5' ITR, and the second ITR sequence is a 3' ITR.
2. The isolated nucleic acid of claim 1, wherein each of the two ITR sequences is a wild-type AAV2 ITR sequence.
3. The isolated nucleic acid of claim 1, wherein each of the two ITR sequences comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct.
4. The isolated nucleic acid of claim 1, wherein at least one of the ITR sequences comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.
5. The isolated nucleic acid of claim 1, wherein the ITR sequence positioned 5' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct, and the ITR sequence positioned 3' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.
6. The isolated nucleic acid of claim 1, wherein the nucleic acid sequence of the 5' ITR is nucleotides 1 – 145 of SEQ ID NO: 69 and the nucleic acid sequence of the 3' ITR is nucleotides 4041 – 4185 of SEQ ID NO: 69.

7. The isolated nucleic acid of any one of claims 1 to 6, further comprising a TRY region between the 5' ITR and the expression construct, wherein the TRY region has the sequence set forth in SEQ ID NO: 28.

5 8. A recombinant adeno-associated virus (rAAV) vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and a transgene encoding a PGRN protein flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITR) sequences, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 68, wherein the first ITR
10 sequence is a 5' ITR, and the second ITR sequence is a 3' ITR.

9. The rAAV vector of claim 8, wherein each of the two ITR sequences is a wild-type AAV2 ITR sequence.

15 10. The rAAV vector of claim 8, wherein each of the two ITR sequences comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct.

11. The rAAV vector of claim 8, wherein at least one of the ITR sequences comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence
20 relative to the expression construct.

12. The rAAV vector of claim 8, wherein the ITR sequence positioned 5' relative to the expression construct comprises a "D" region (SEQ ID NO: 27) that is proximal to the expression construct, and the ITR sequence positioned 3' relative to the expression construct
25 comprises a "D" region (SEQ ID NO: 27) positioned on the outside of the ITR sequence relative to the expression construct.

13. The rAAV vector of claim 8, wherein the nucleic acid sequence of the 5' ITR is nucleotides 1 – 145 of SEQ ID NO: 69 and the nucleic acid sequence of the 3' ITR is
30 nucleotides 4041 – 4185 of SEQ ID NO: 69.

14. The rAAV vector of any one of claims 8 to 13, further comprising a TRY region between the 5' ITR and the expression construct, wherein the TRY region has the sequence set forth in SEQ ID NO: 28.

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15. A rAAV comprising:
(i) an AAV capsid protein; and
(ii) the rAAV vector of any one of claims 8-14.
16. The rAAV of claim 15, wherein the AAV capsid protein is AAV9 capsid protein.
- 10
17. A rAAV vector comprising a nucleic acid comprising, in 5' to 3' order:
(a) a 5' AAV ITR;
(b) a CMV enhancer;
(c) a CBA promoter;
(d) a transgene encoding a PGRN protein, wherein the transgene comprises the nucleotide sequence of SEQ ID NO: 68;
15
(e) a WPRE;
(f) a Bovine Growth Hormone polyA signal tail; and
(g) a 3' AAV ITR.
- 20
18. A rAAV comprising:
(i) an AAV capsid protein; and
(ii) the rAAV vector of claim 17.
- 25
19. The rAAV of claim 18, wherein the AAV capsid protein is AAV9 capsid protein.
20. A plasmid comprising the rAAV vector of any one of claims 8-14 and 17.
- 30
21. A Baculovirus vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide sequence set forth in SEQ ID NO: 68.
22. A cell comprising:
(i) a first vector encoding one or more adeno-associated virus rep proteins and/or one or more adeno-associated virus cap proteins; and

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(ii) a second vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide sequence set forth in SEQ ID NO: 68.

5 23. The cell of claim 22, wherein the first vector is a plasmid and the second vector is a plasmid.

 24. The cell of claim 22 or 23, wherein the cell is a mammalian cell.

10 25. The cell of claim 24, wherein the mammalian cell is a HEK293 cell.

 26. The cell of claim 22, wherein the first vector is a Baculovirus vector and the second vector is a Baculovirus vector.

15 27. The cell of claim 22 or 26, wherein the cell is an insect cell.

 28. A method of producing the rAAV of any one of claims 15, 16, 18 and 19, the method comprising:

 (i) delivering to a cell a first vector encoding one or more adeno-associated virus rep protein and/or one or more adeno-associated virus cap protein, and a rAAV vector comprising an expression construct comprising a CBA promoter, a CMV enhancer, a WPRE, a Bovine Growth Hormone polyA signal tail, and the nucleotide sequence of SEQ ID NO: 68;

 (ii) culturing the cells under conditions allowing for packaging the rAAV; and

 (iii) harvesting the cultured host cell or culture medium for collection of the
25 rAAV.

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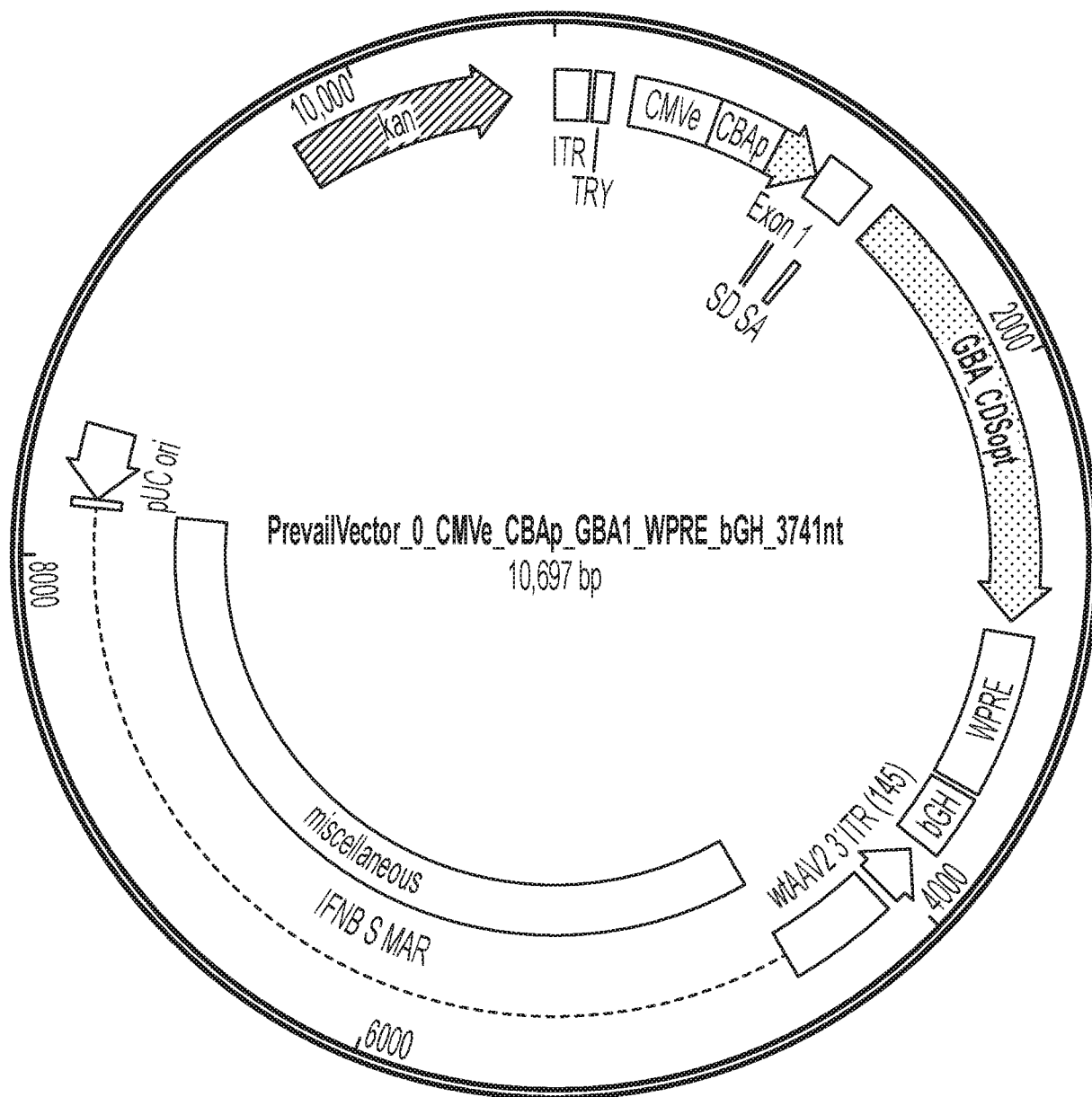


Figure 1

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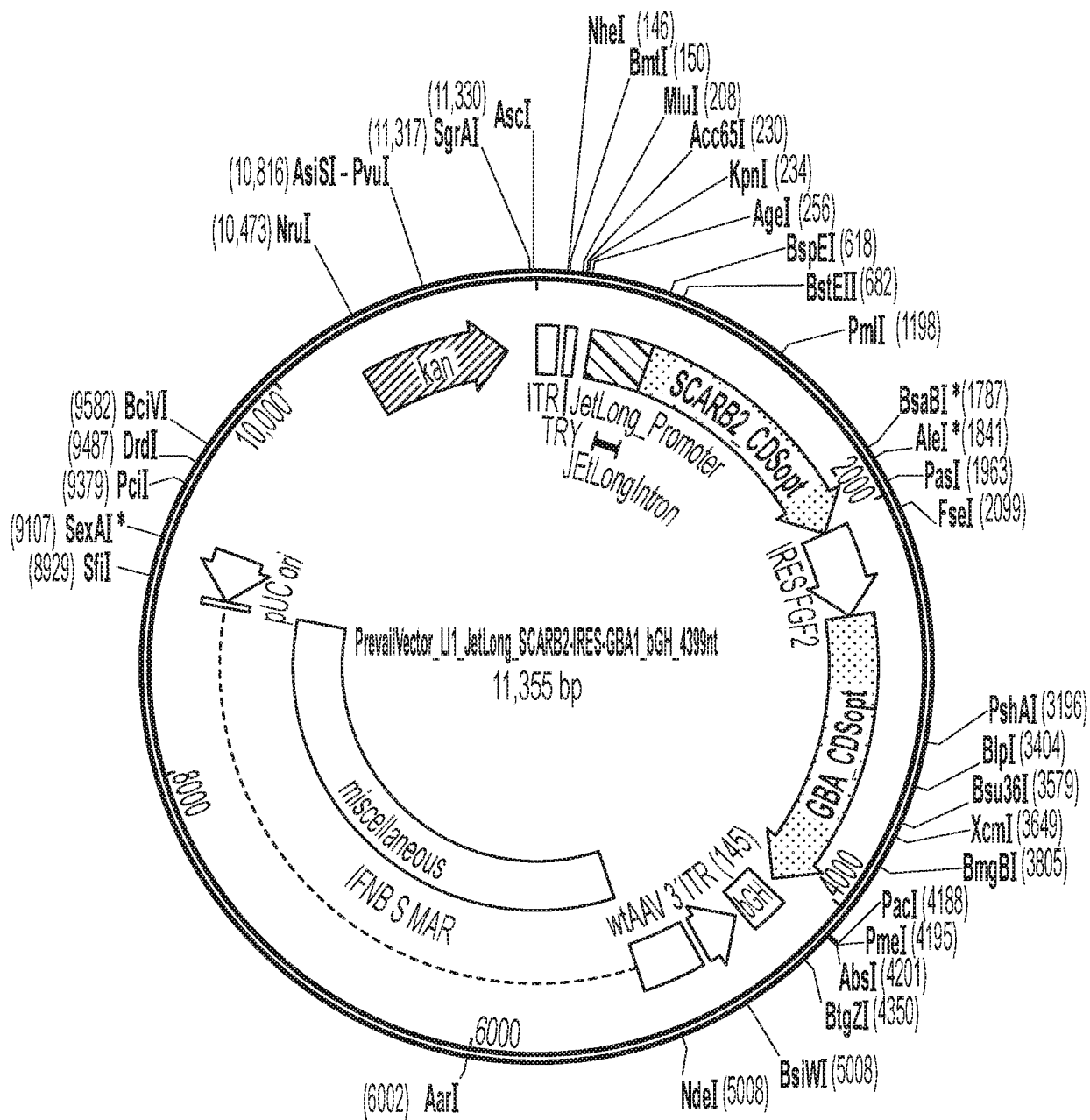


Figure 2

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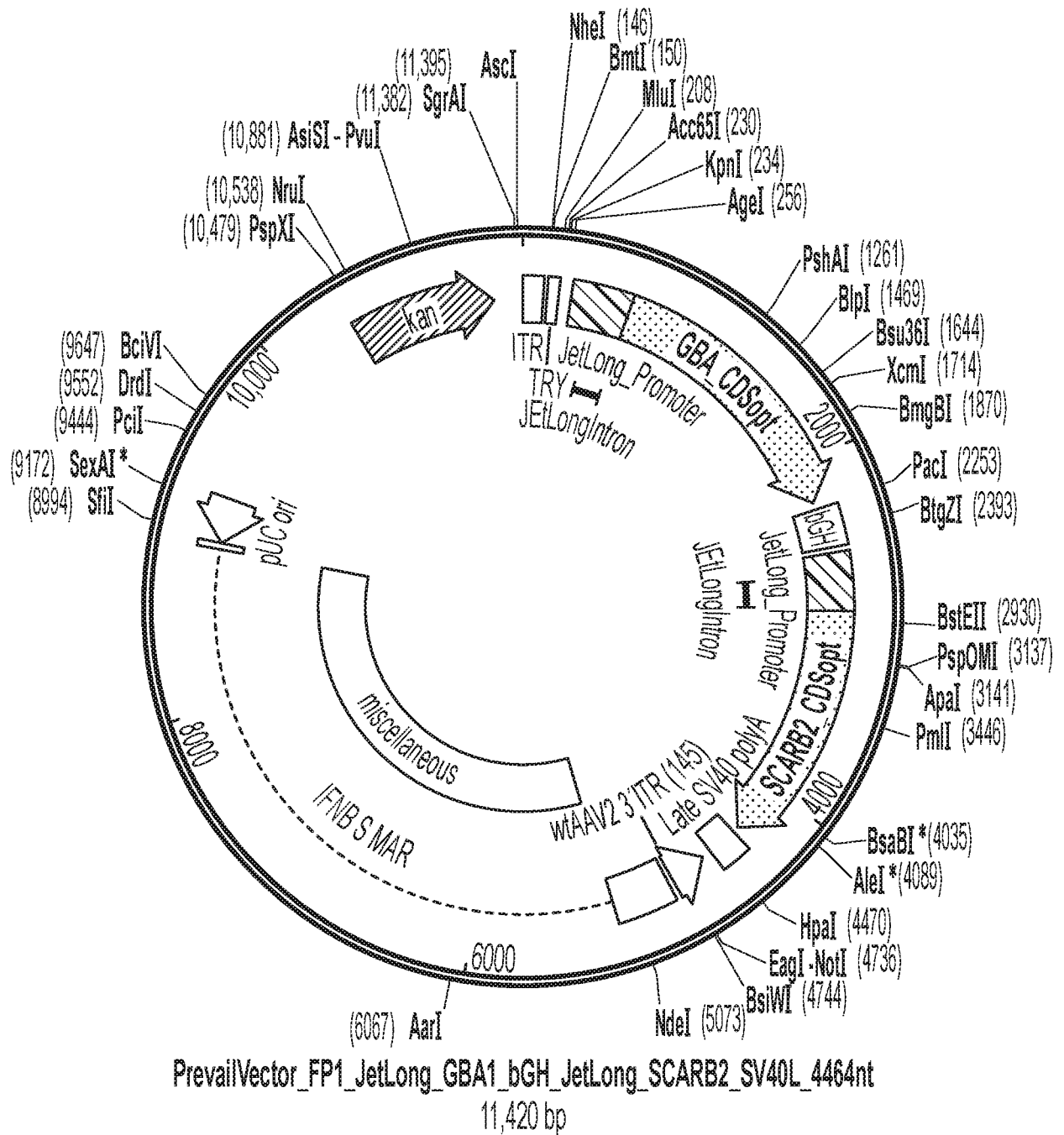


Figure 3

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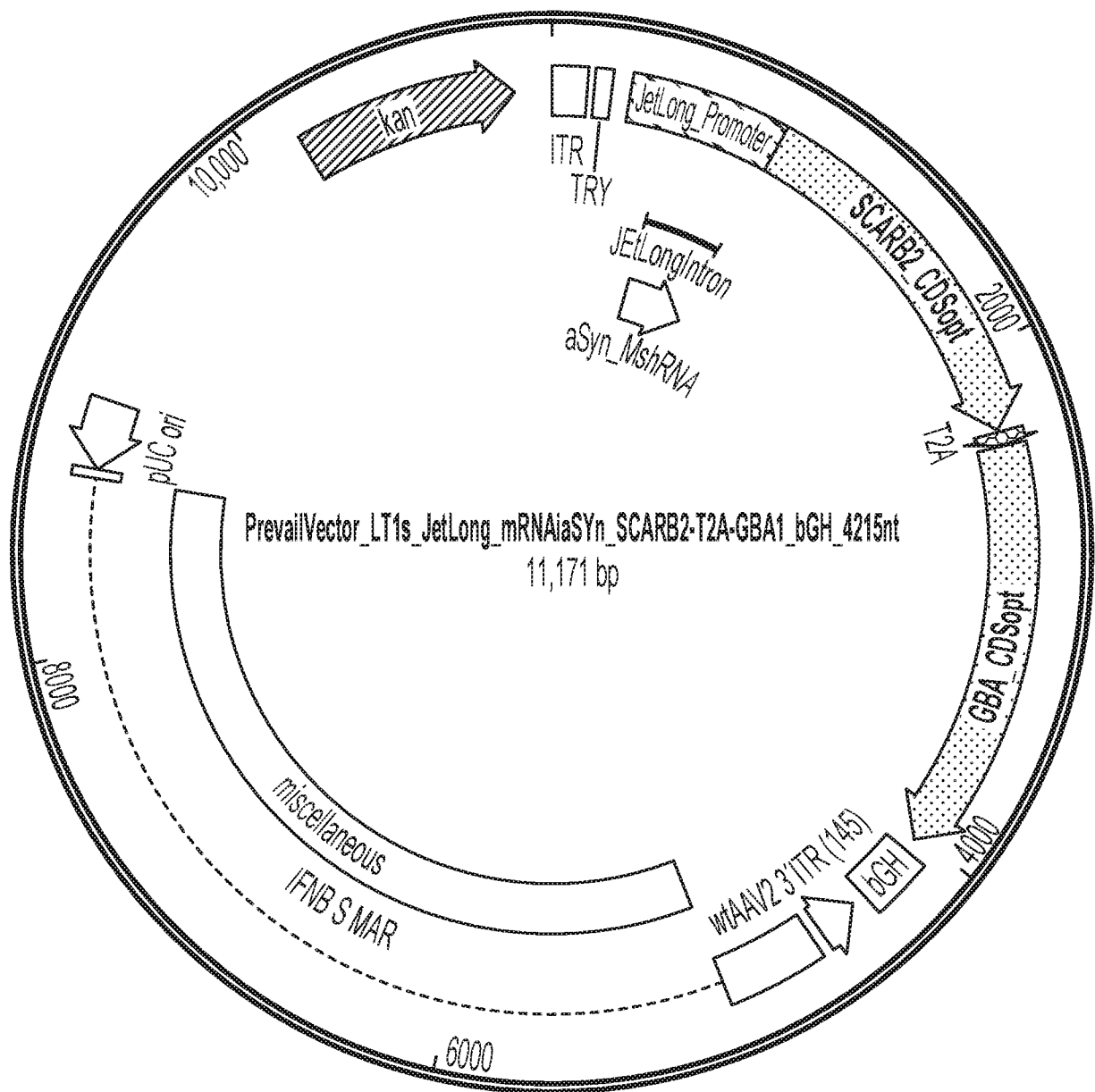


Figure 4

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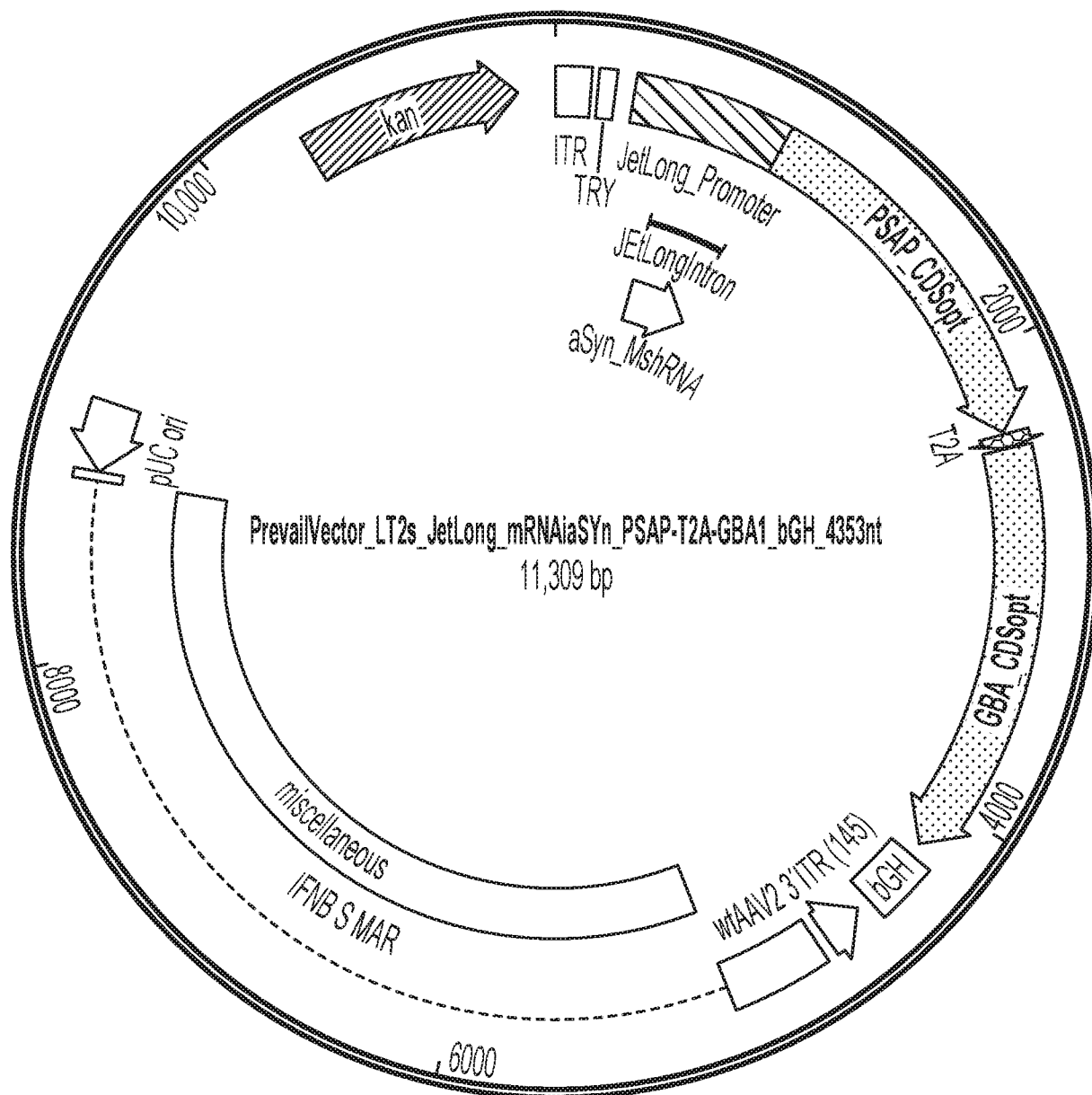


Figure 5

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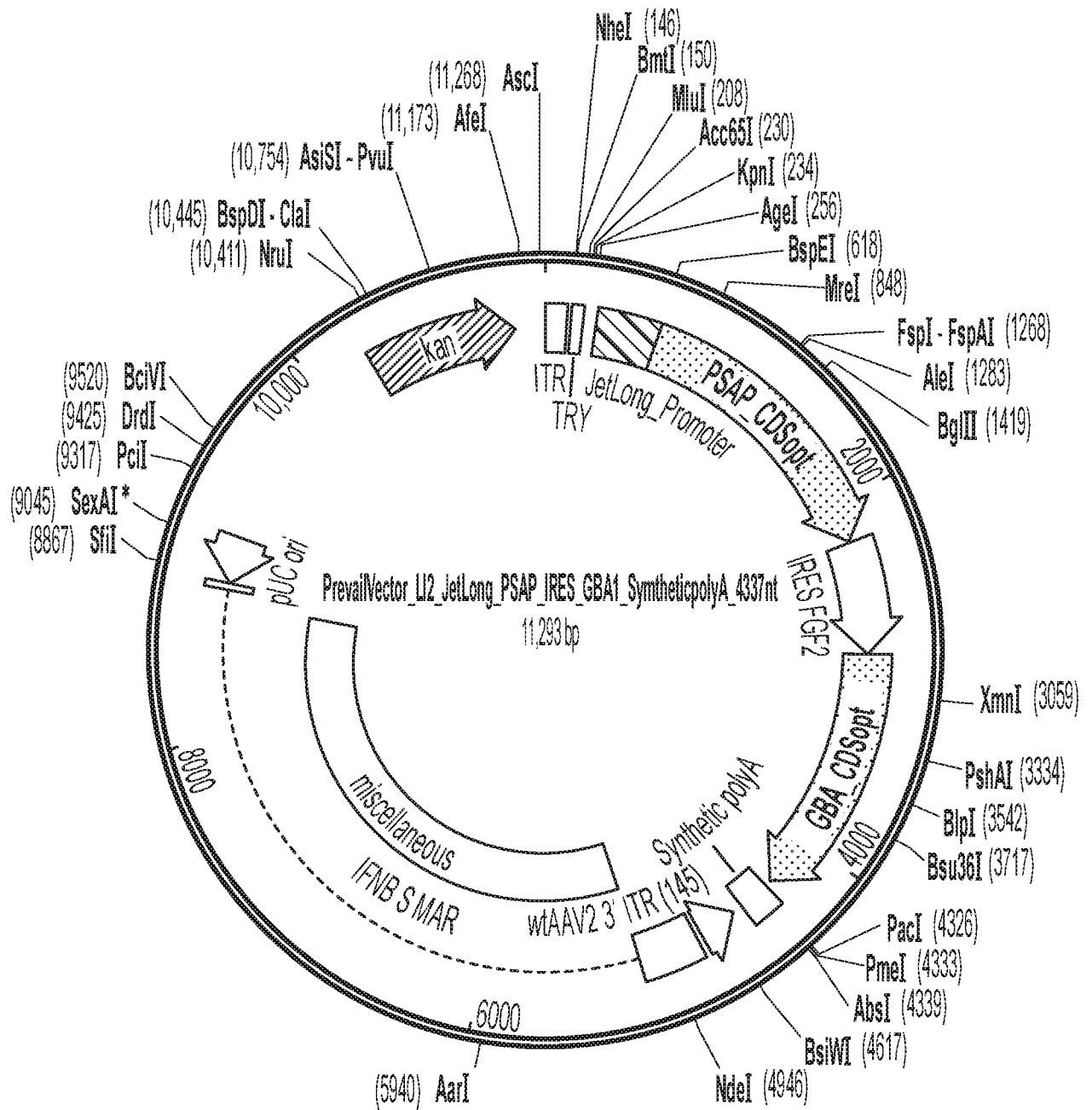


Figure 6

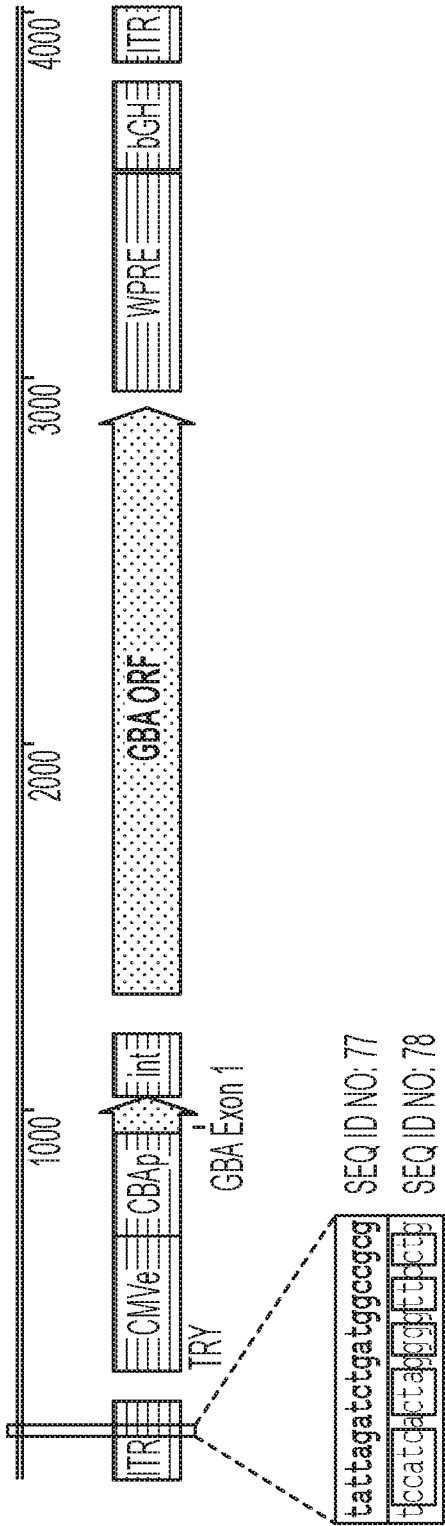


Figure 7

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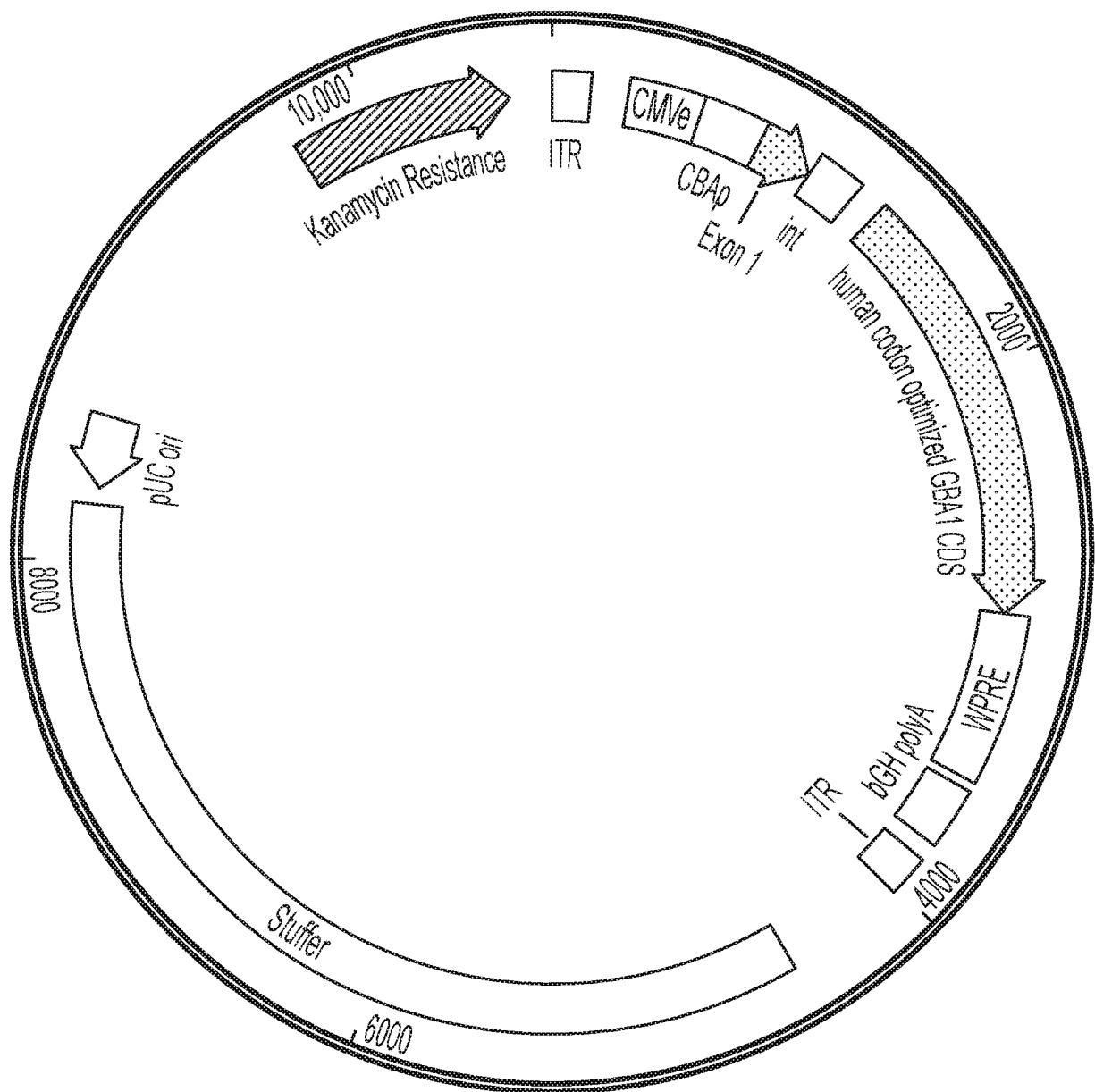


Figure 8

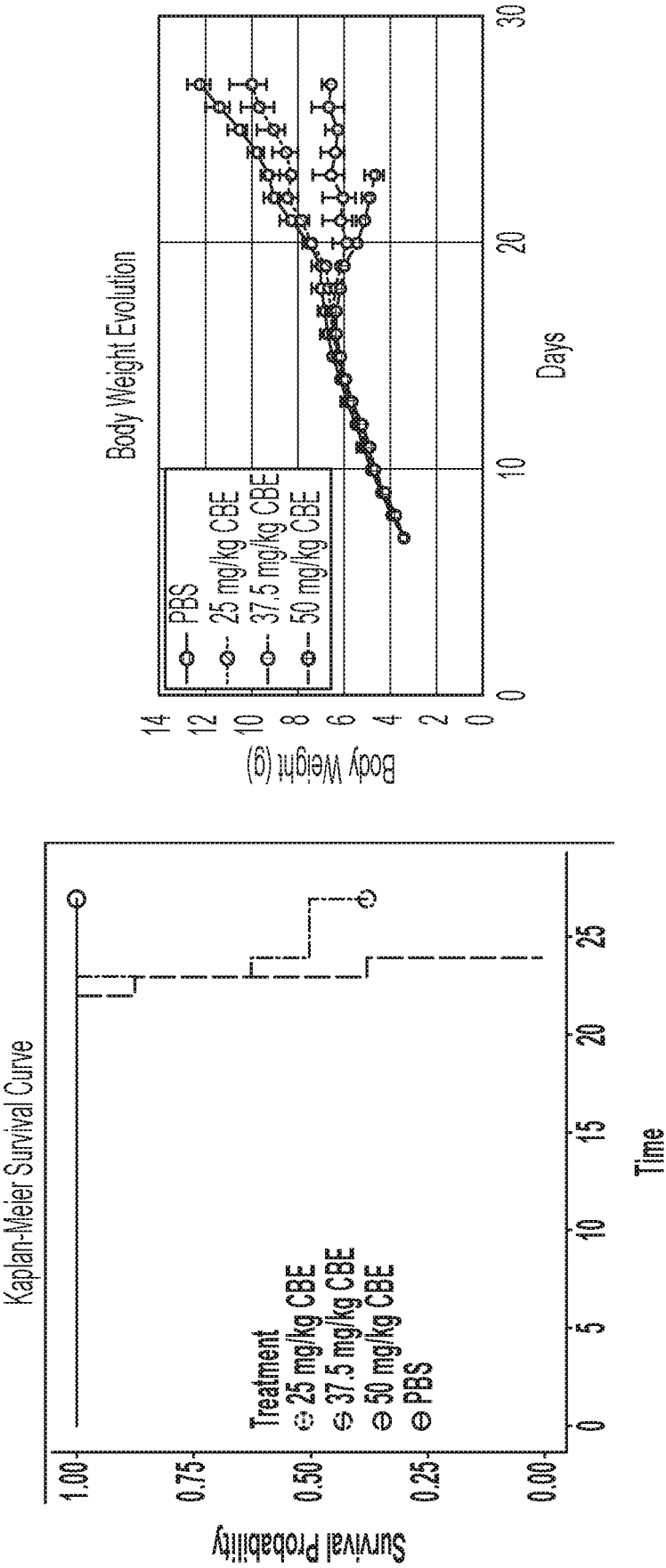


Figure 9

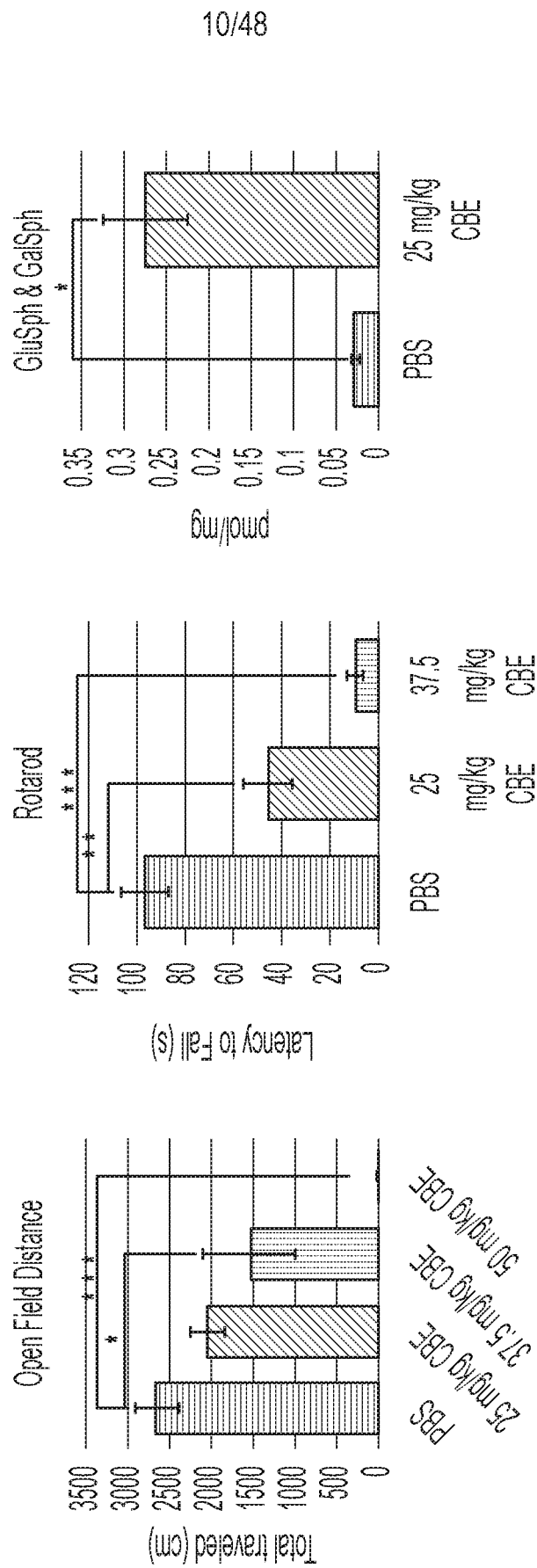


Figure 9 cont.

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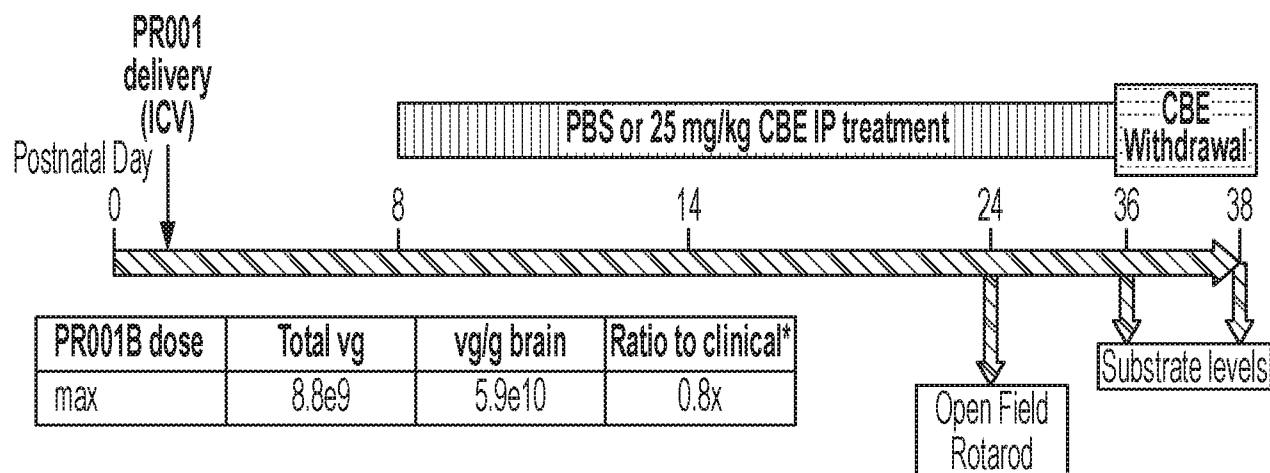


Figure 10

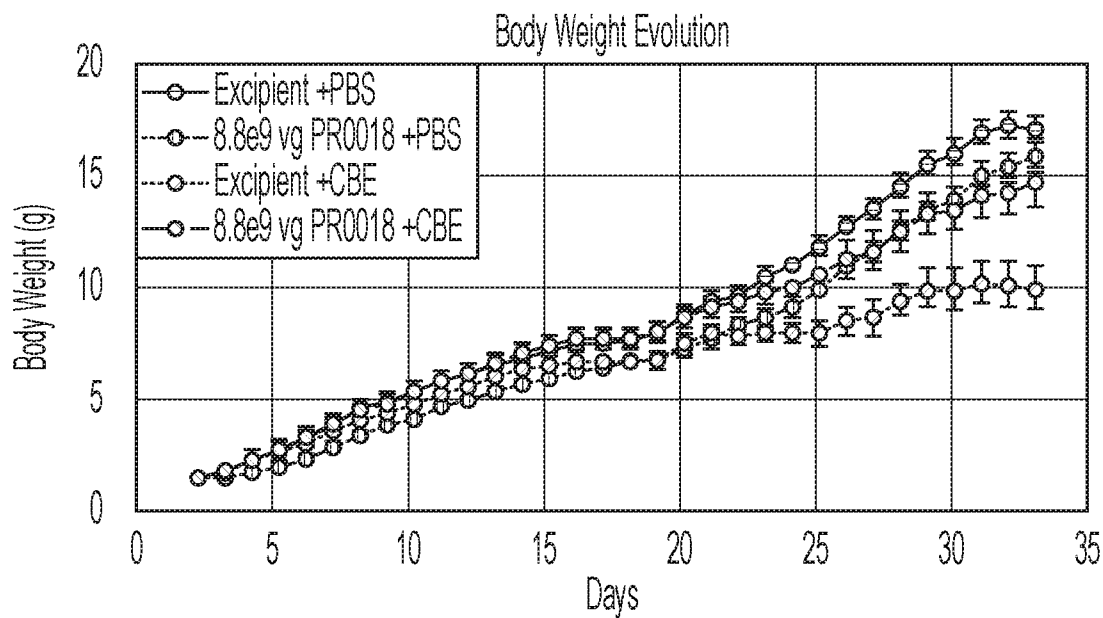


Figure 11

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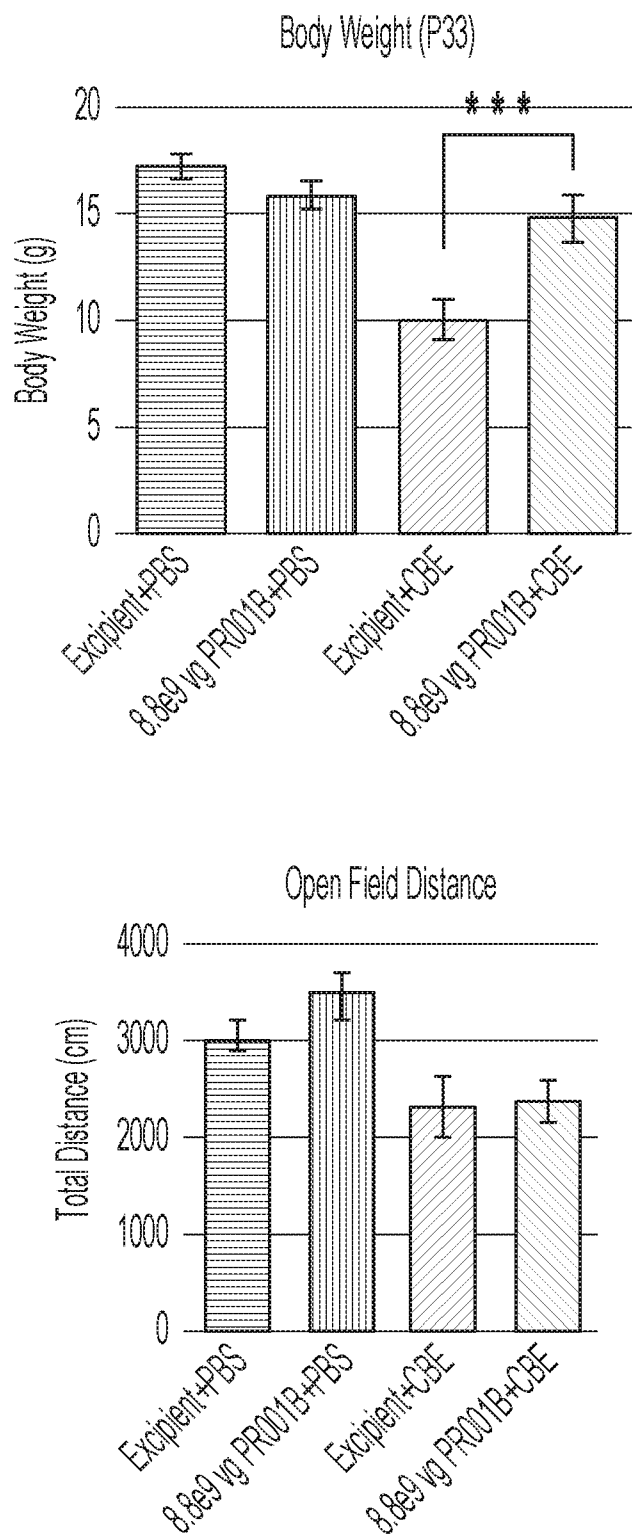


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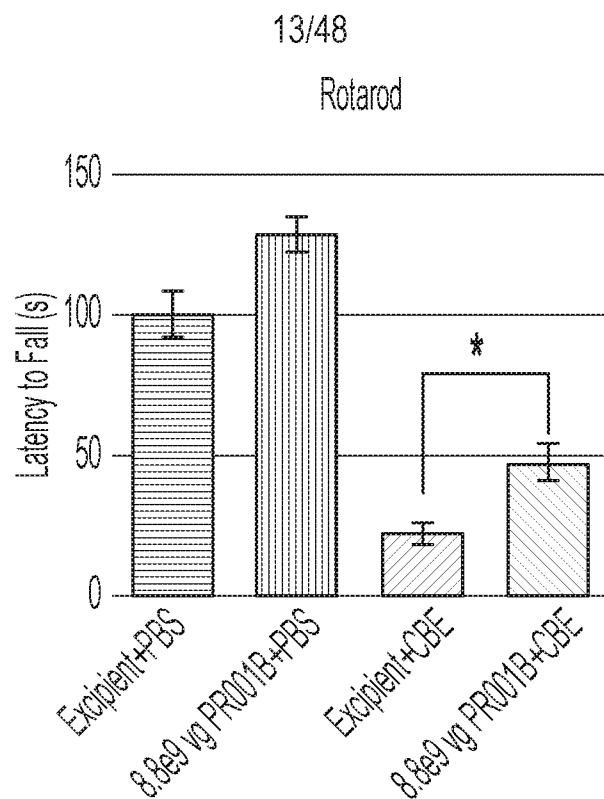


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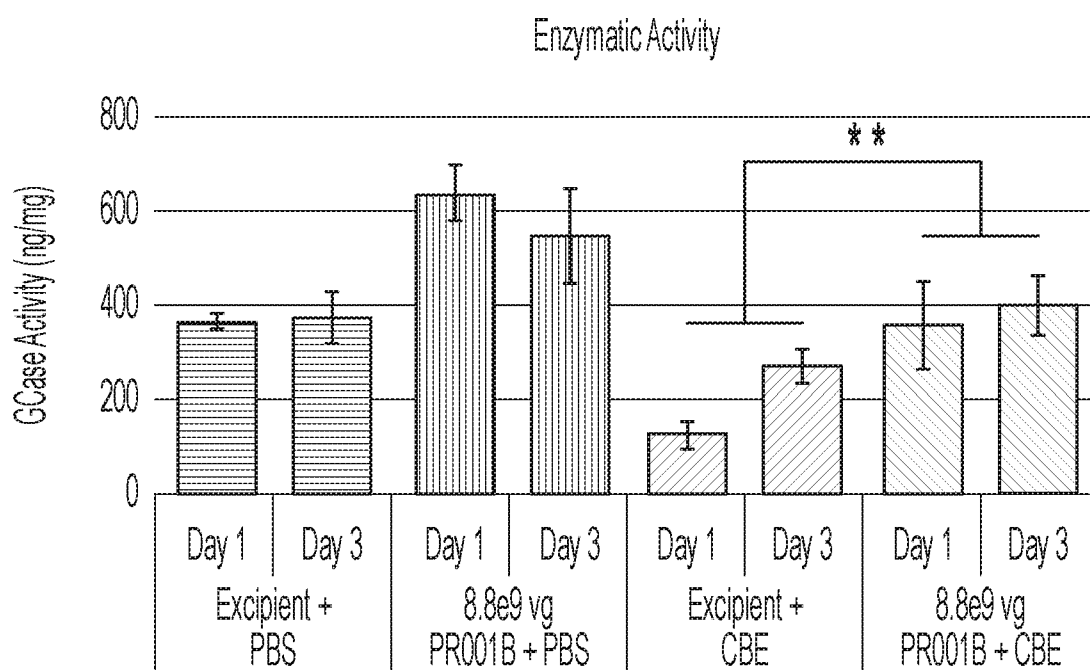


Figure 12

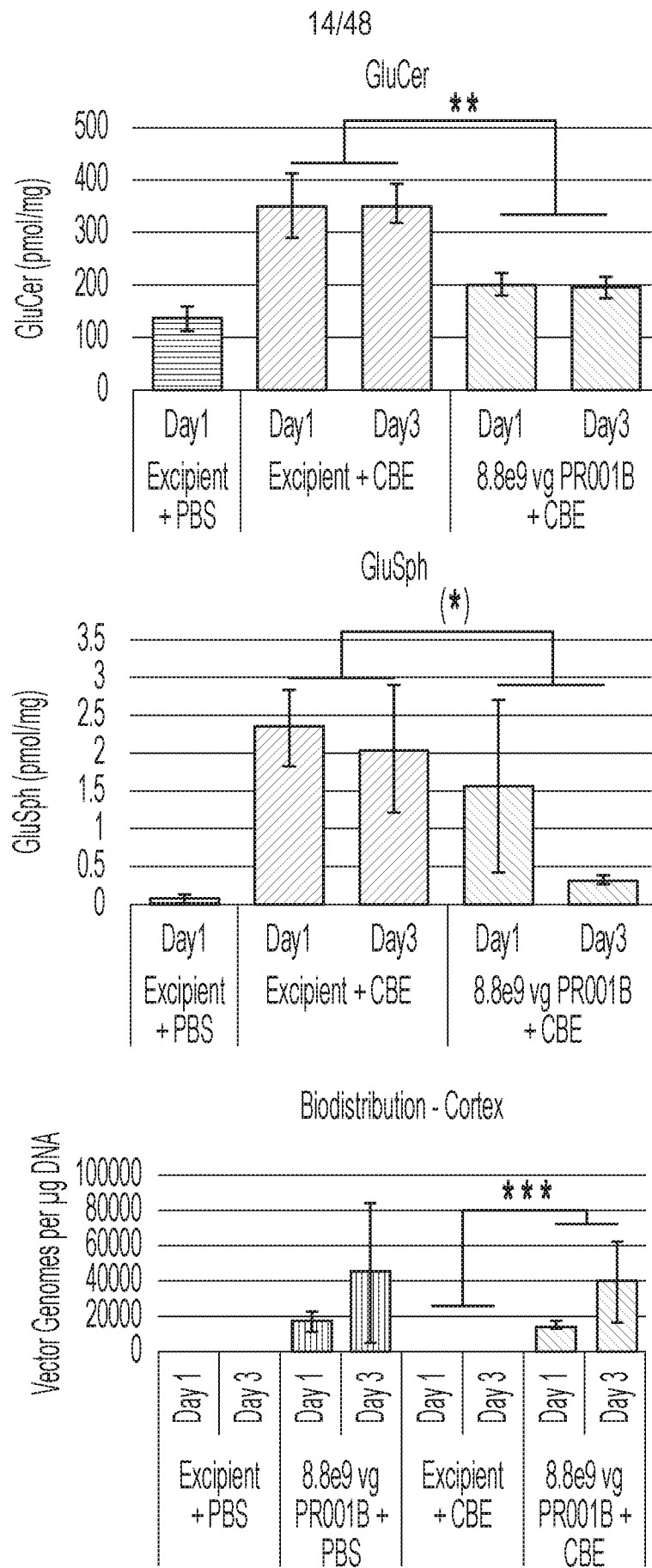


Figure 12 cont.

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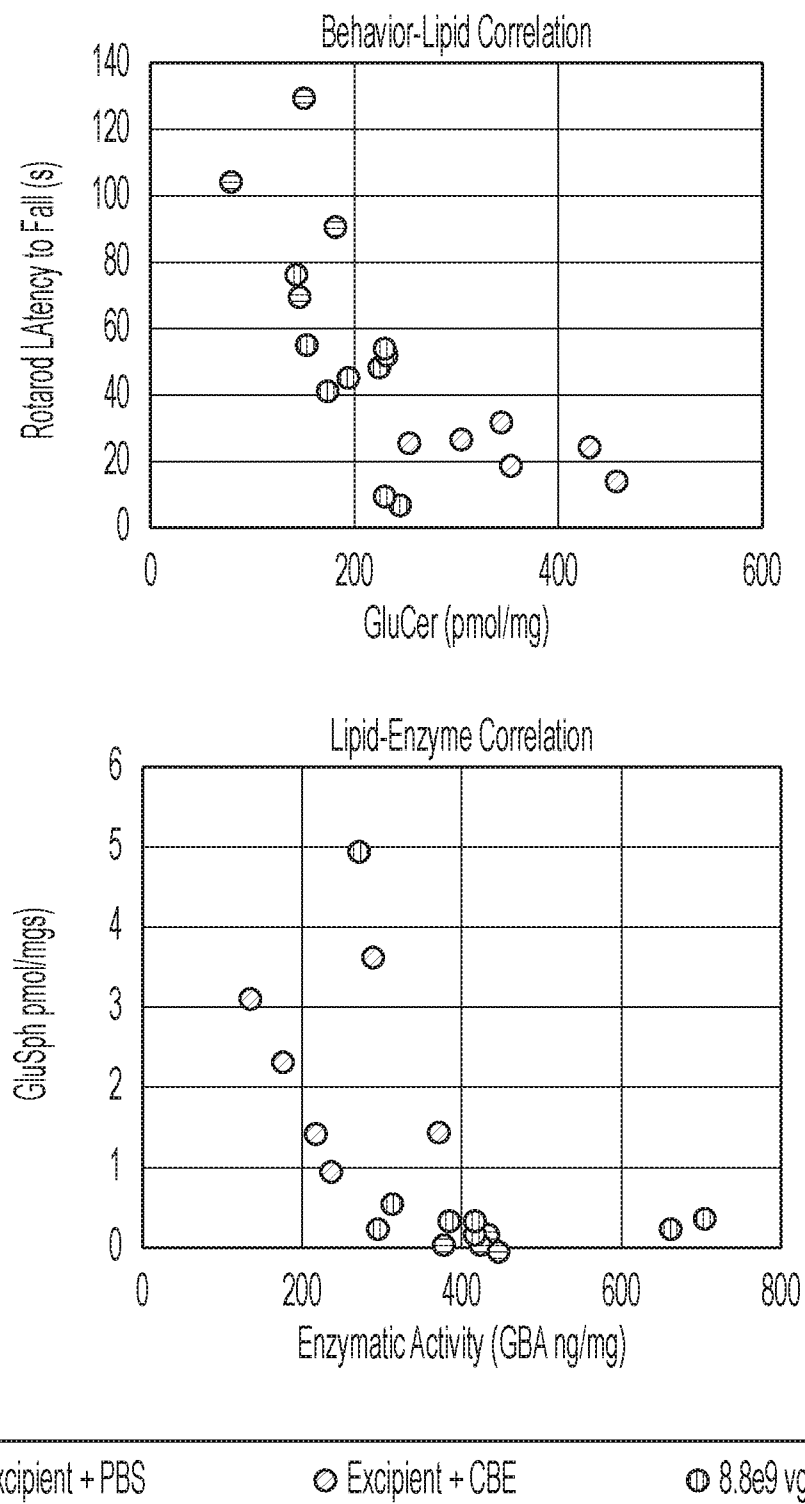


Figure 13

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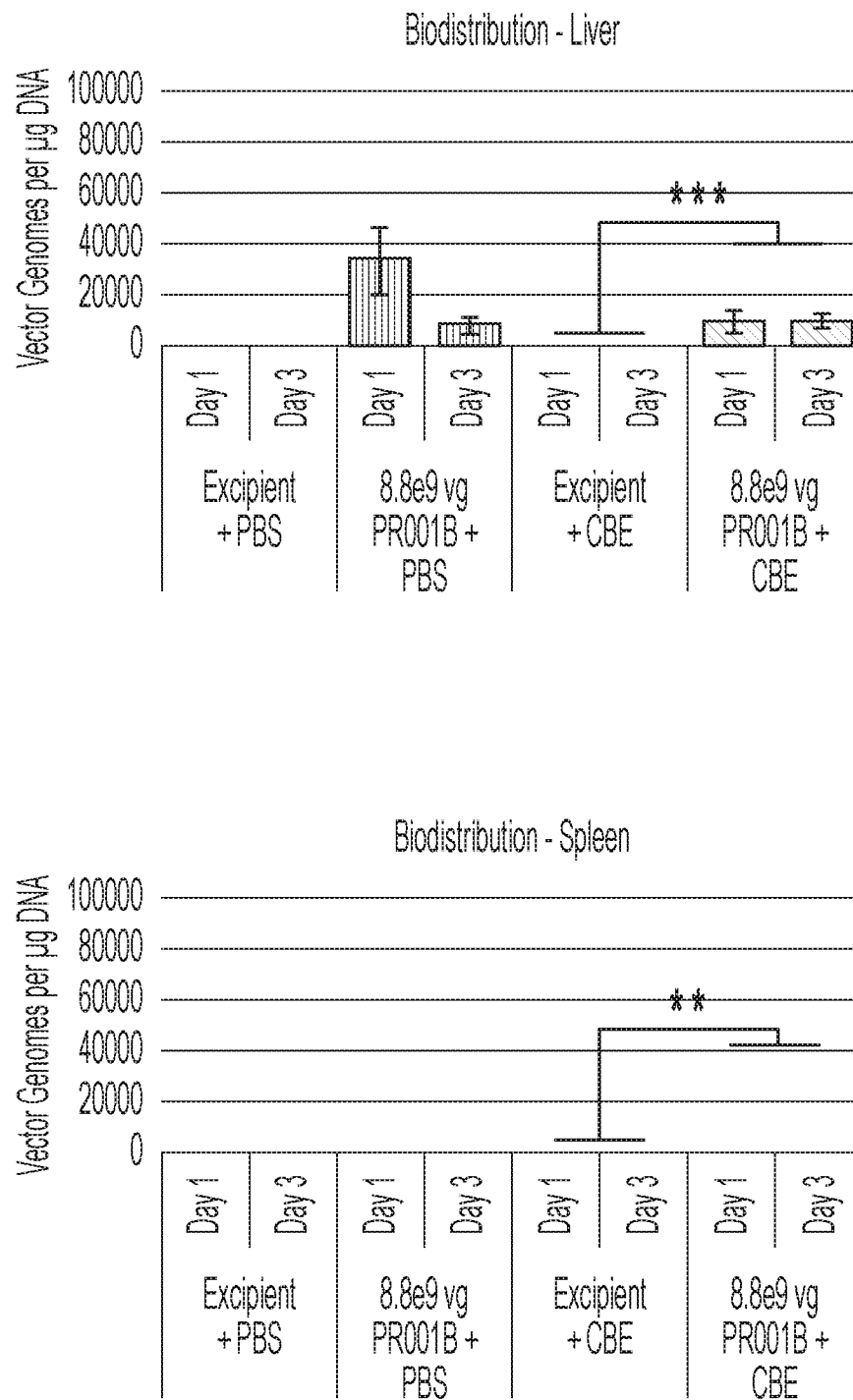


Figure 14

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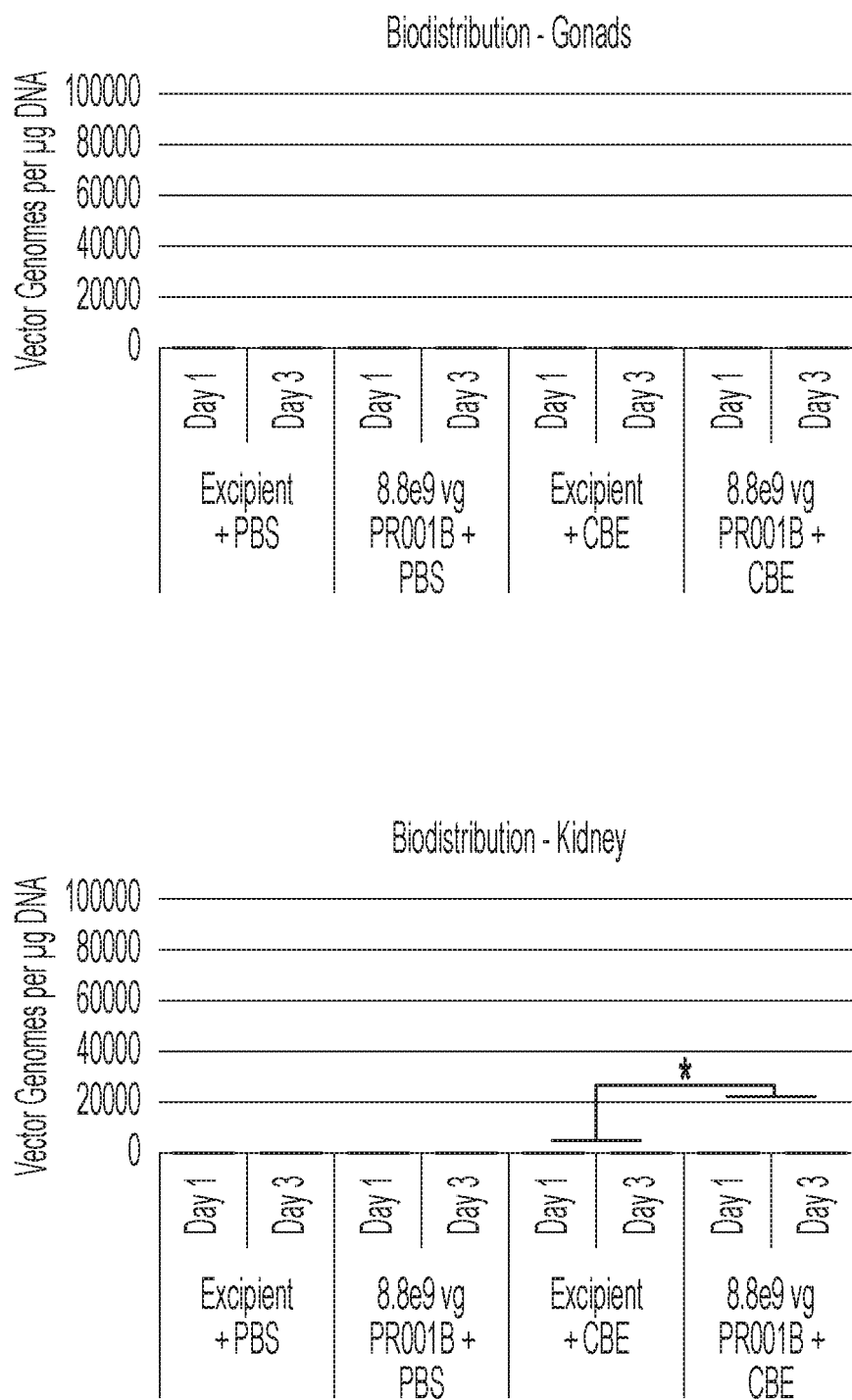


Figure 14 cont.

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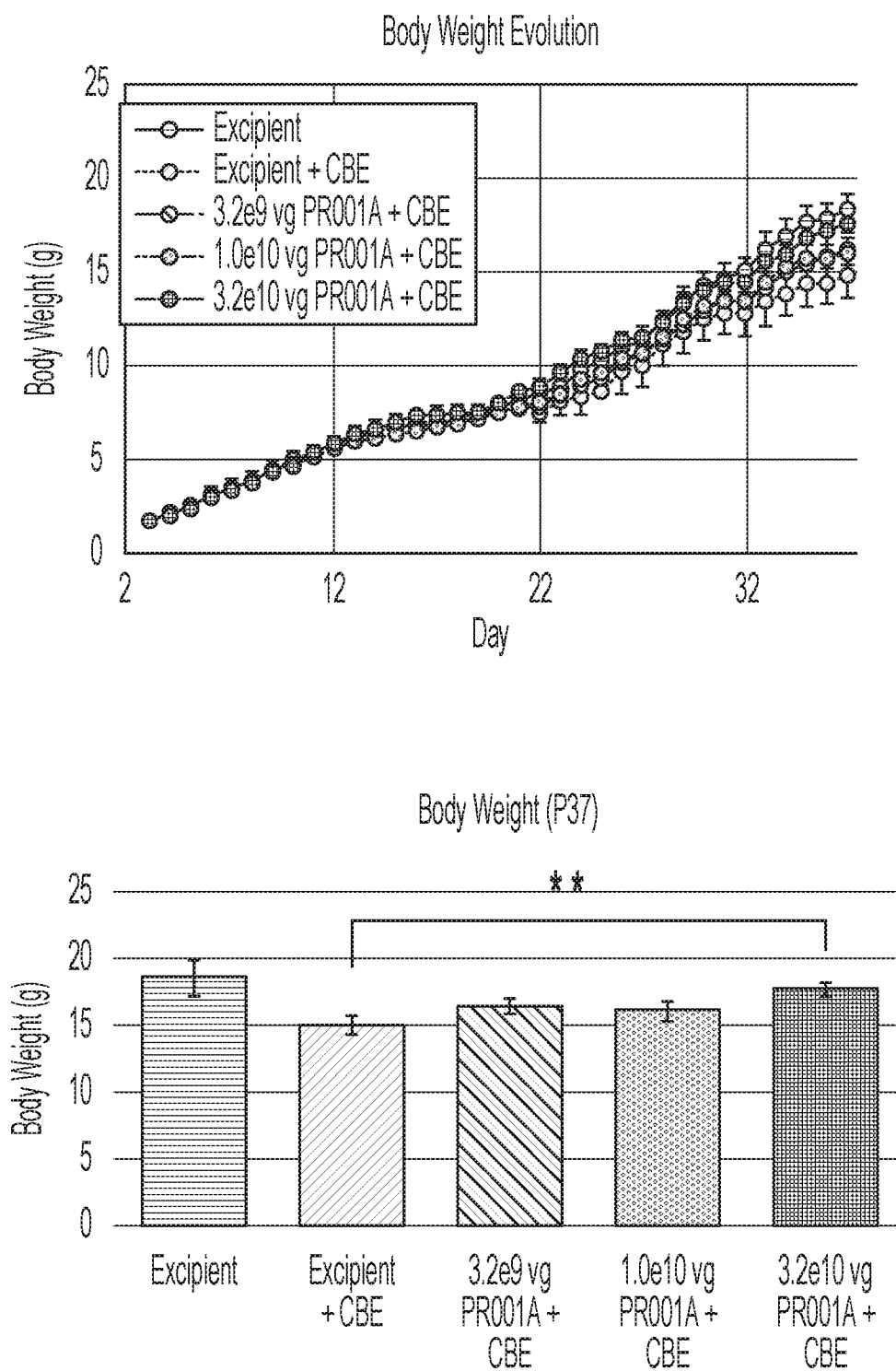


Figure 15

19/48

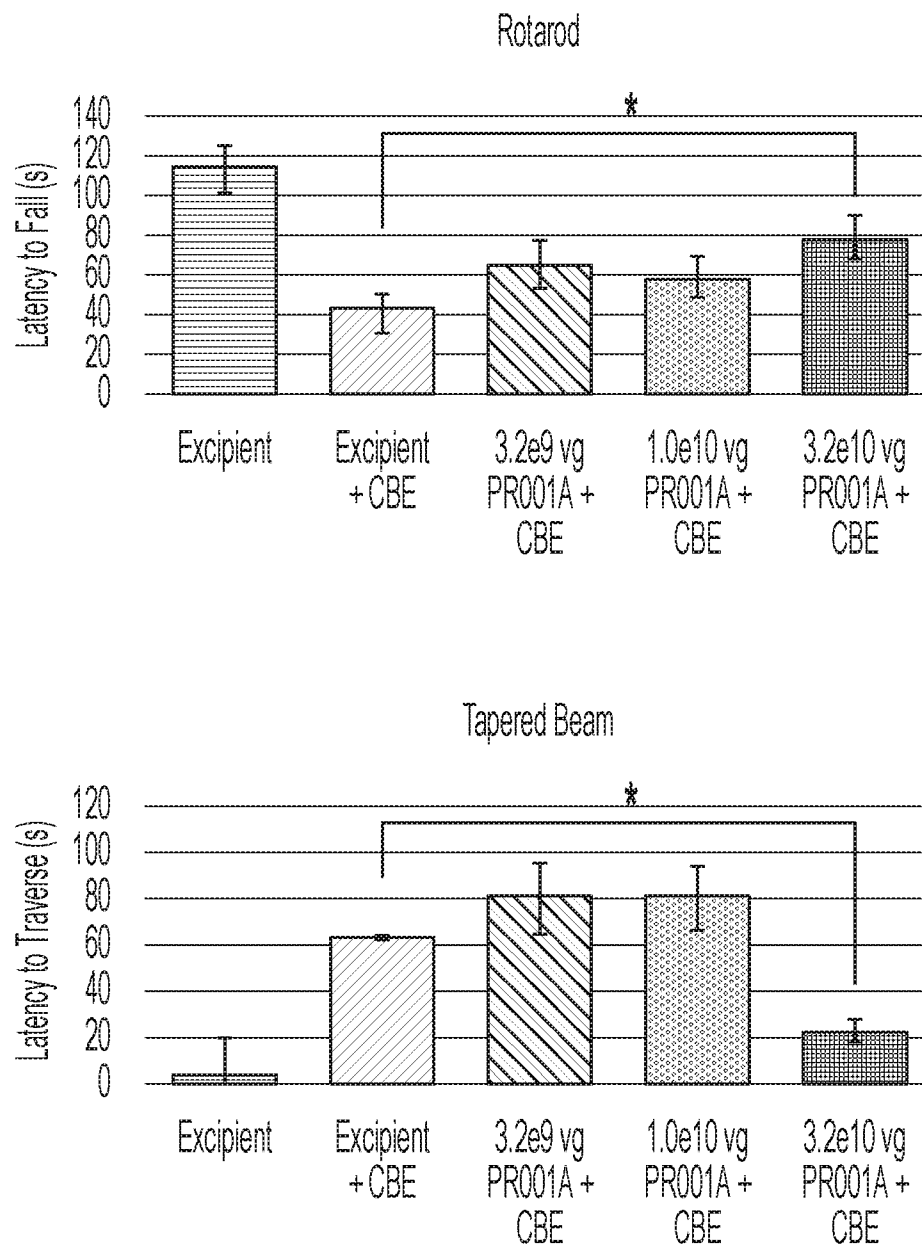


Figure 15 cont.

20/48

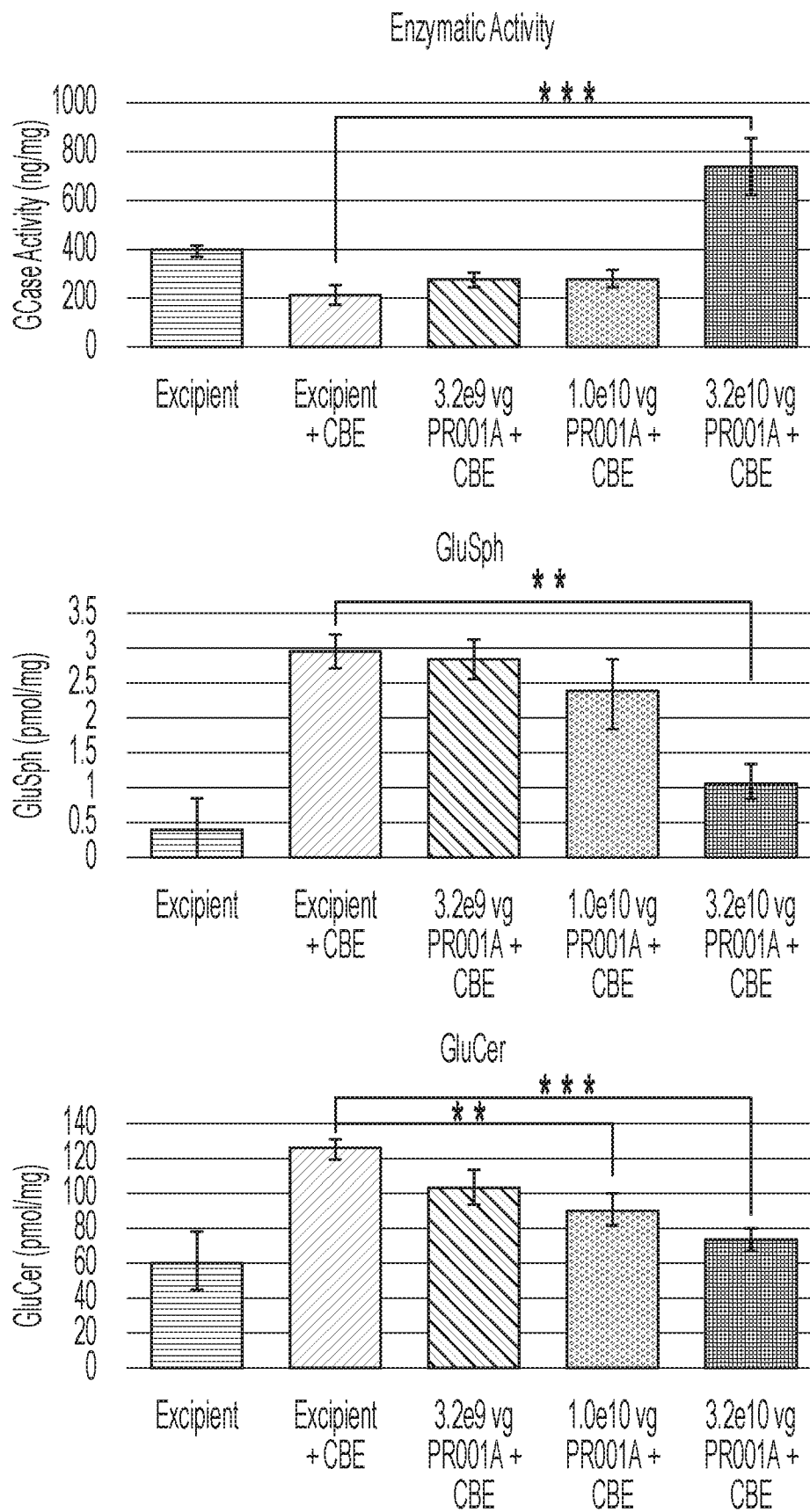


Figure 16

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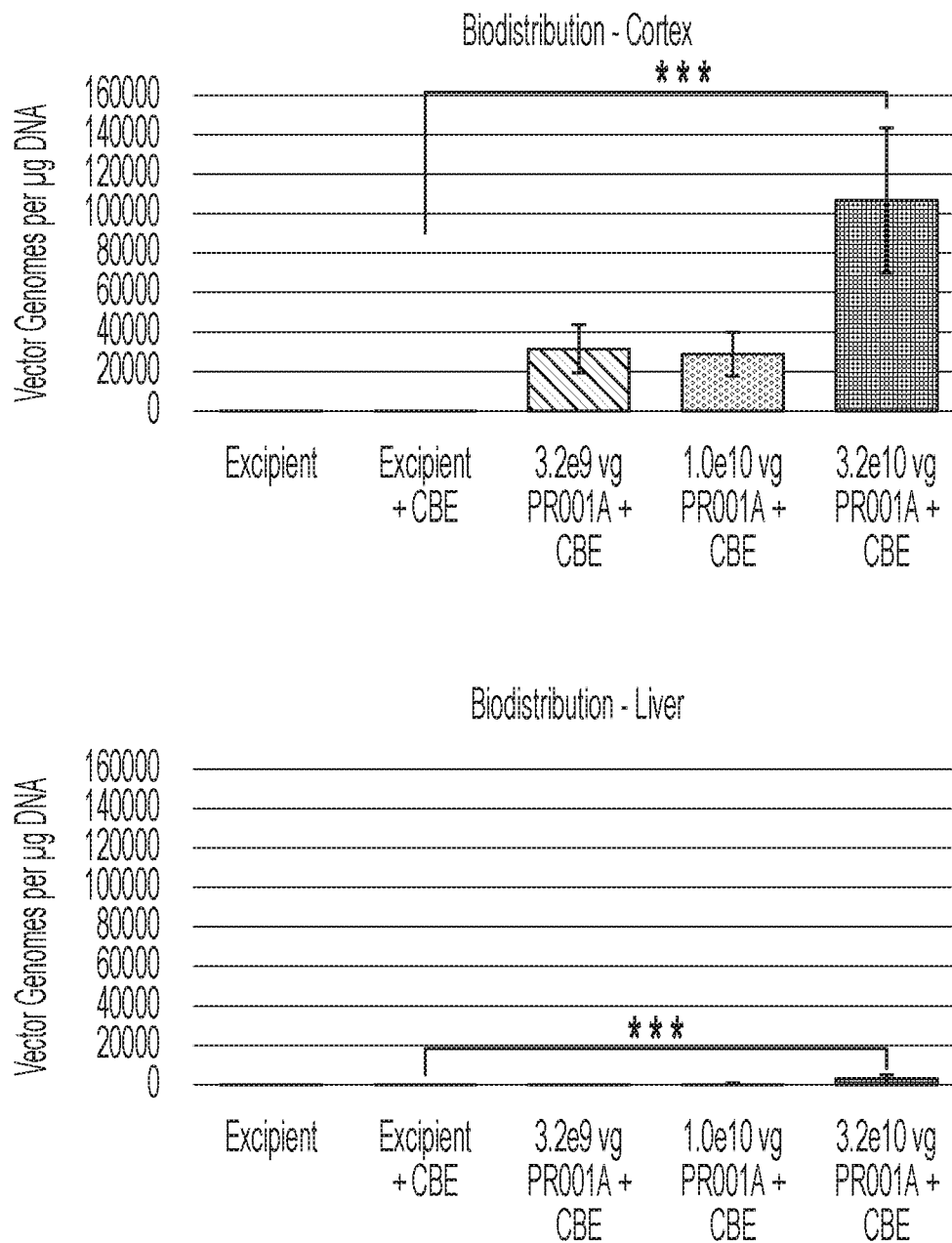


Figure 16 cont.

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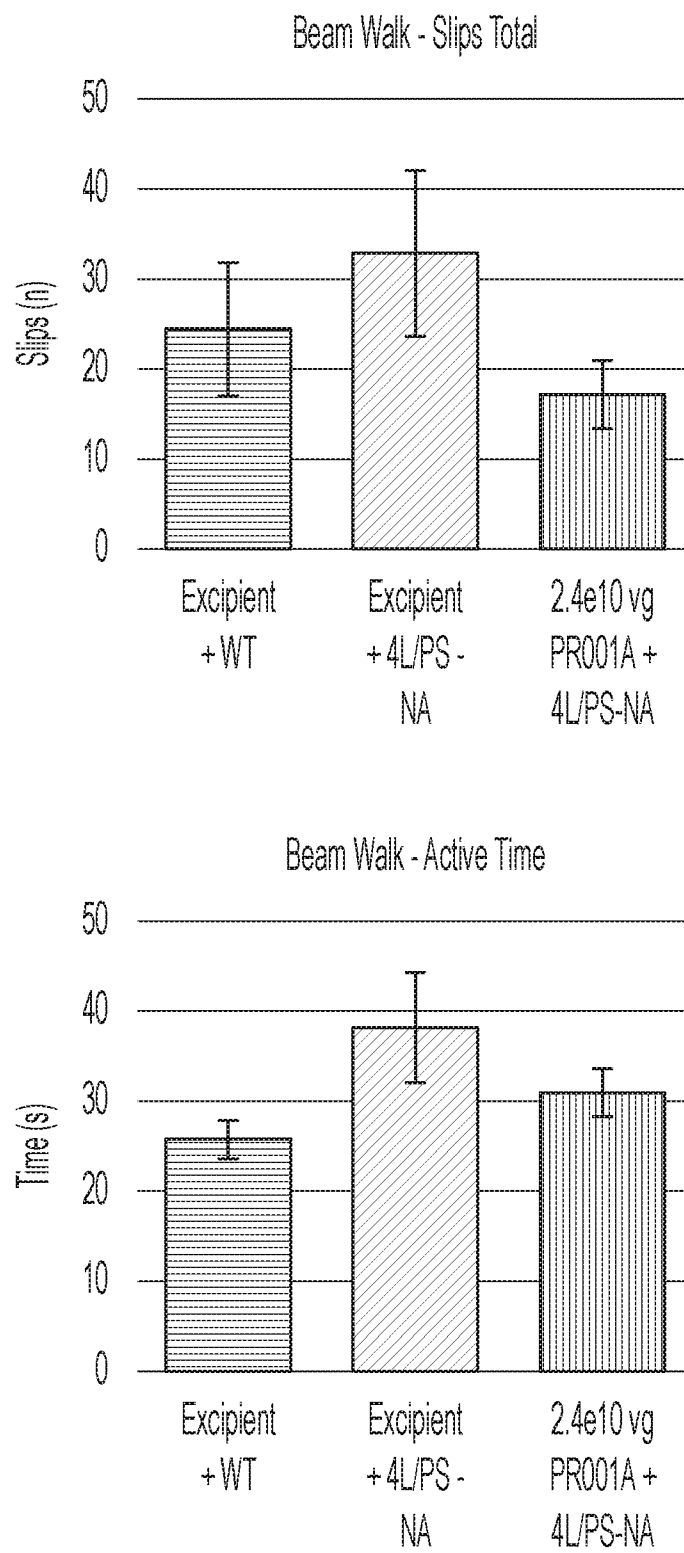


Figure 17

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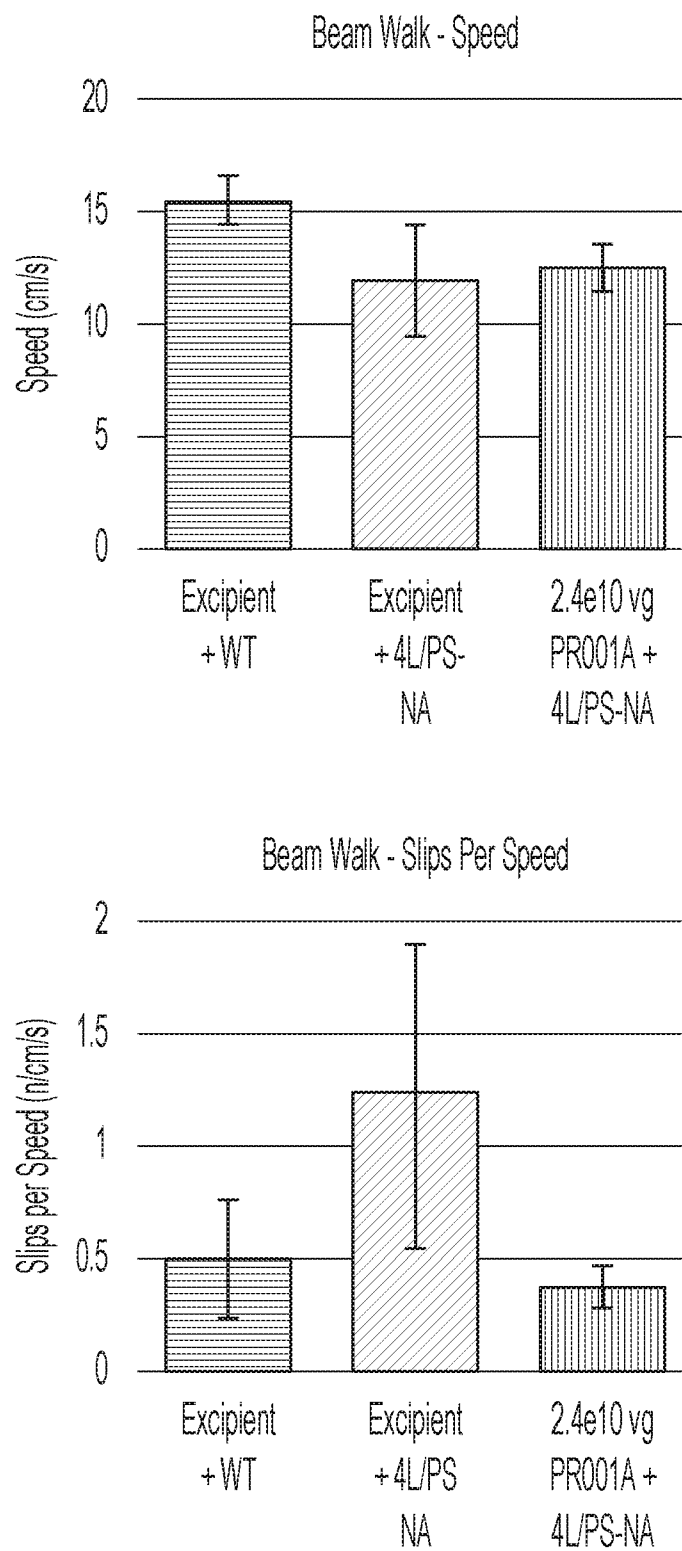


Figure 17 cont.

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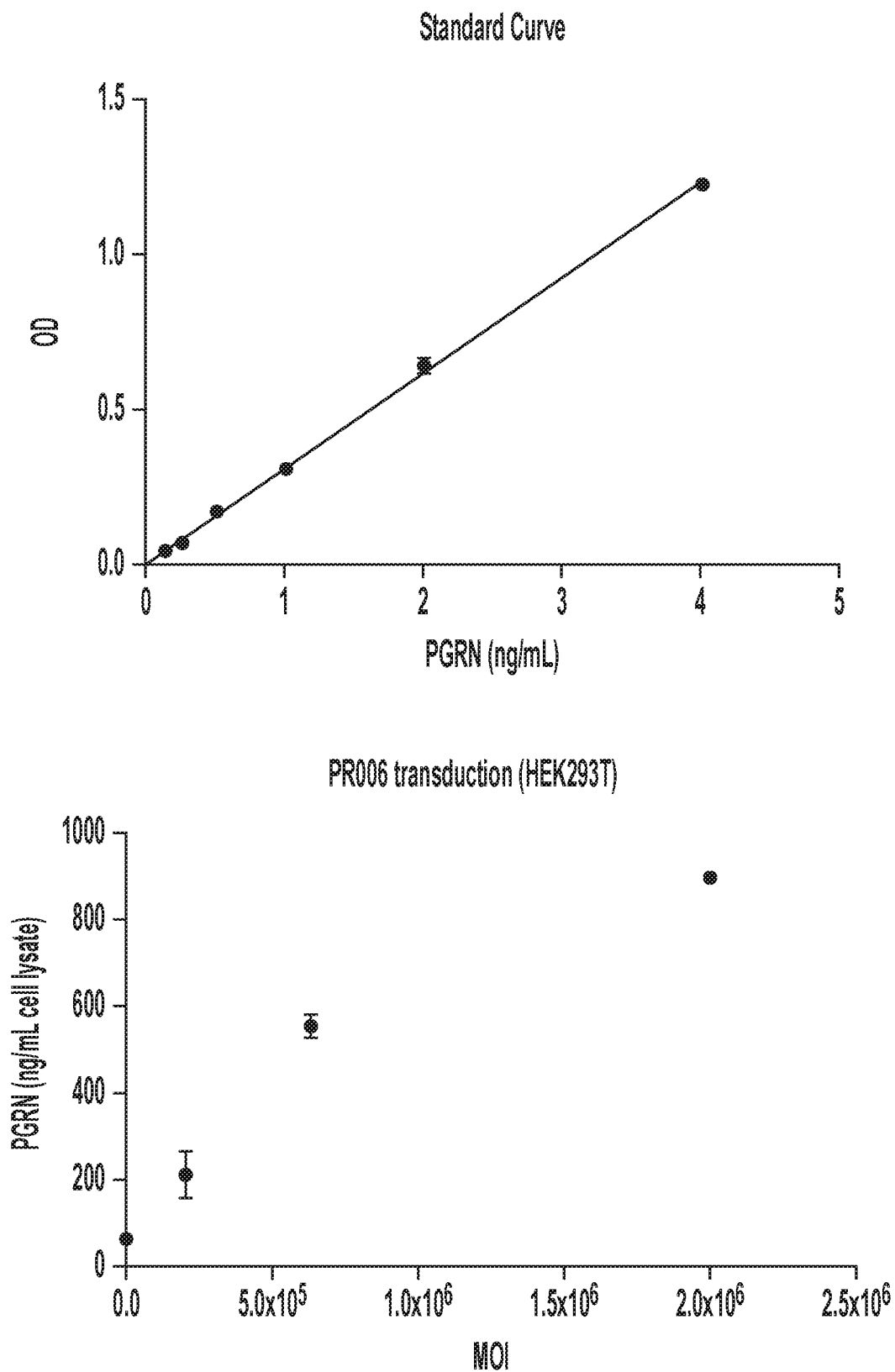


Figure 18

25/48

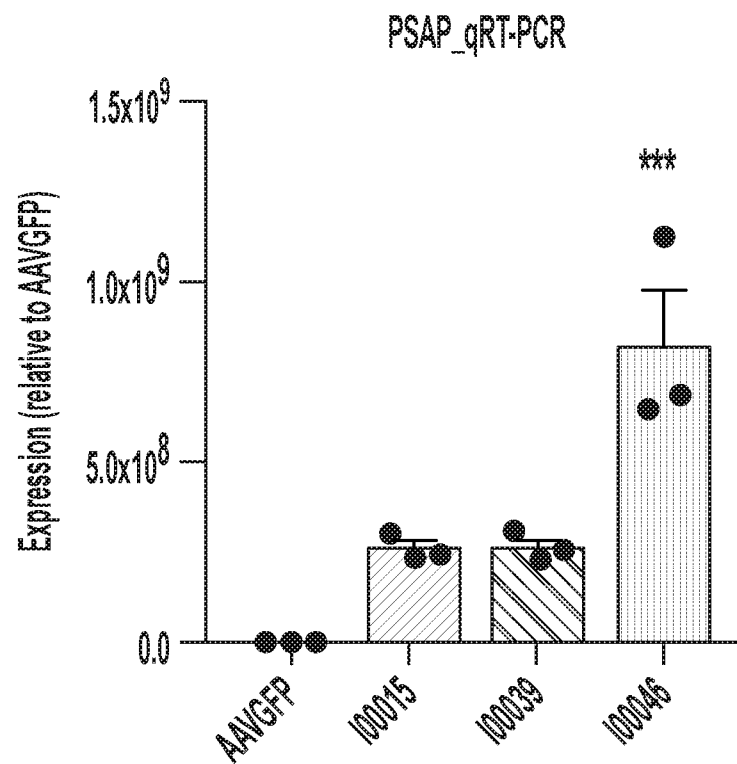


Figure 19

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PSAP_ELISA

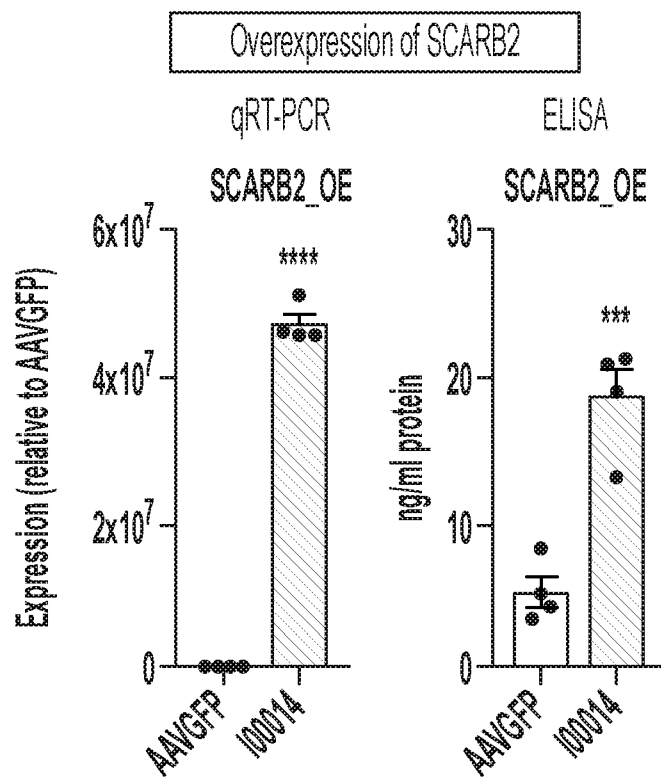
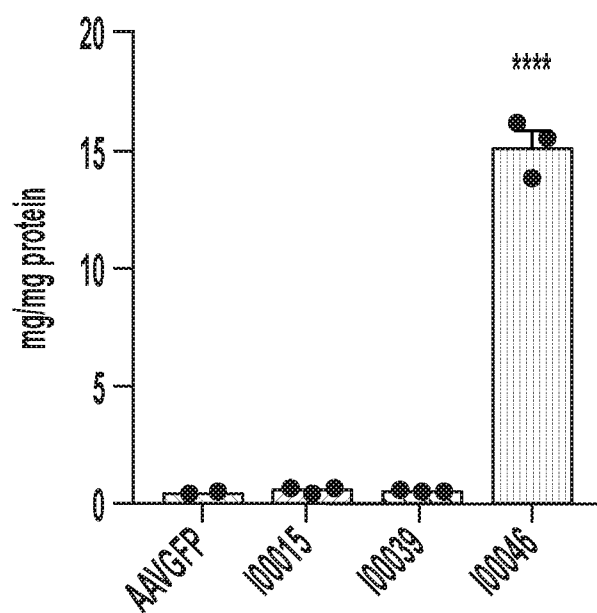


Figure 19 cont.

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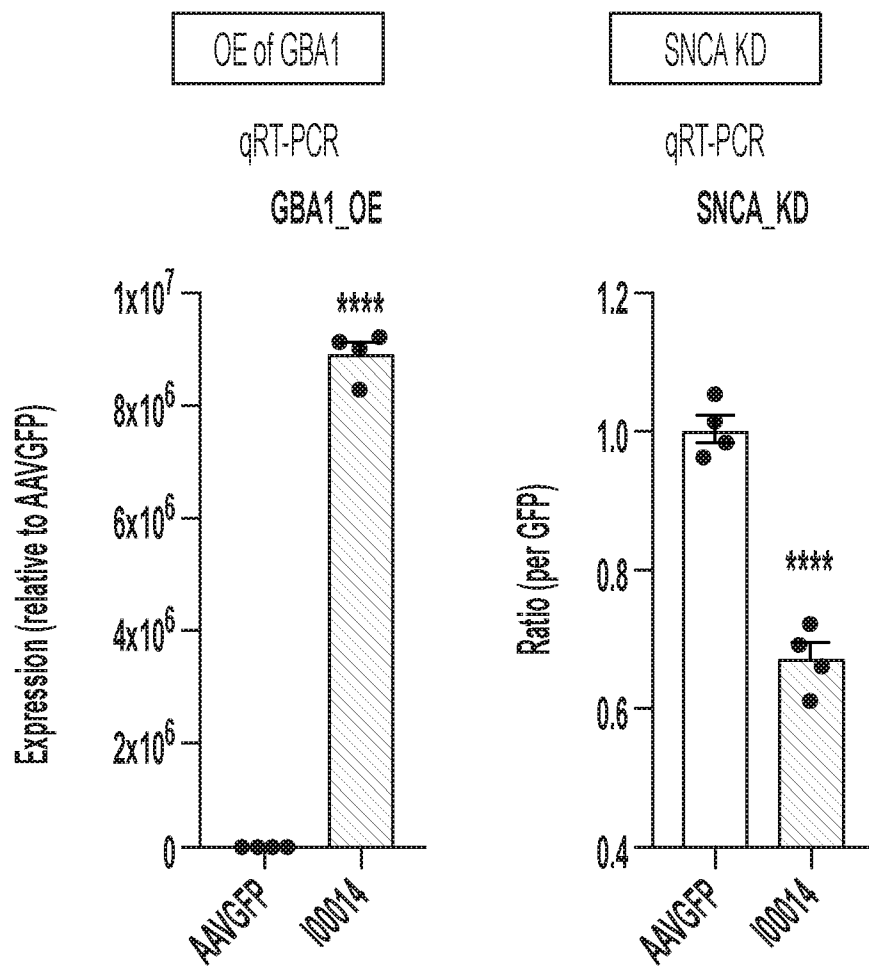


Figure 19 cont.

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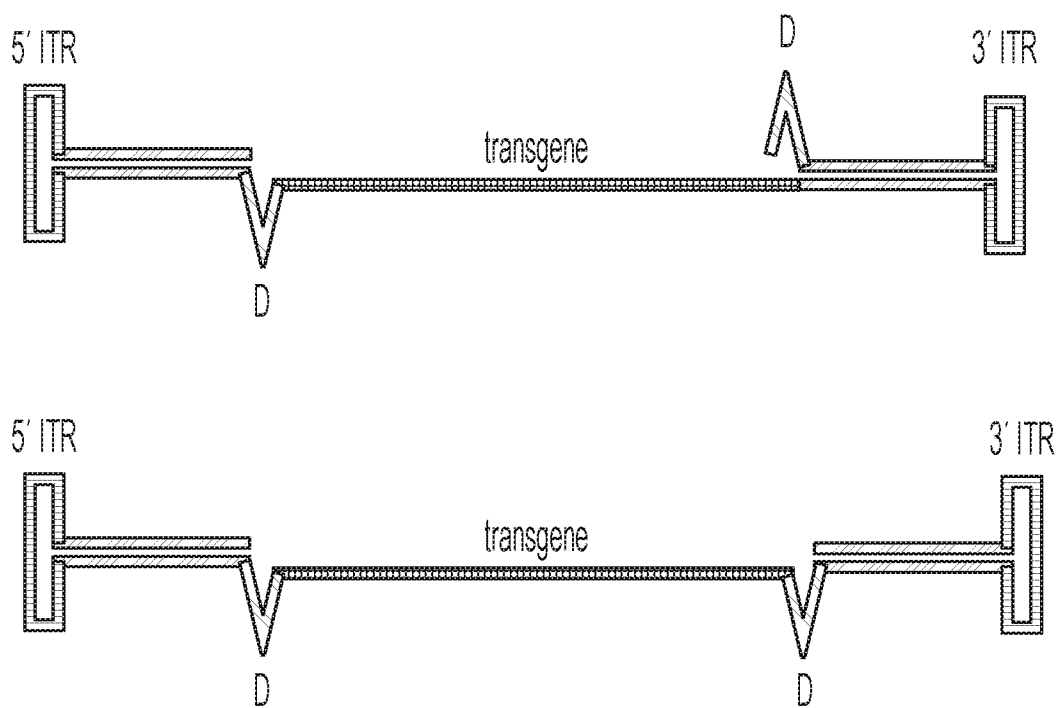


Figure 20

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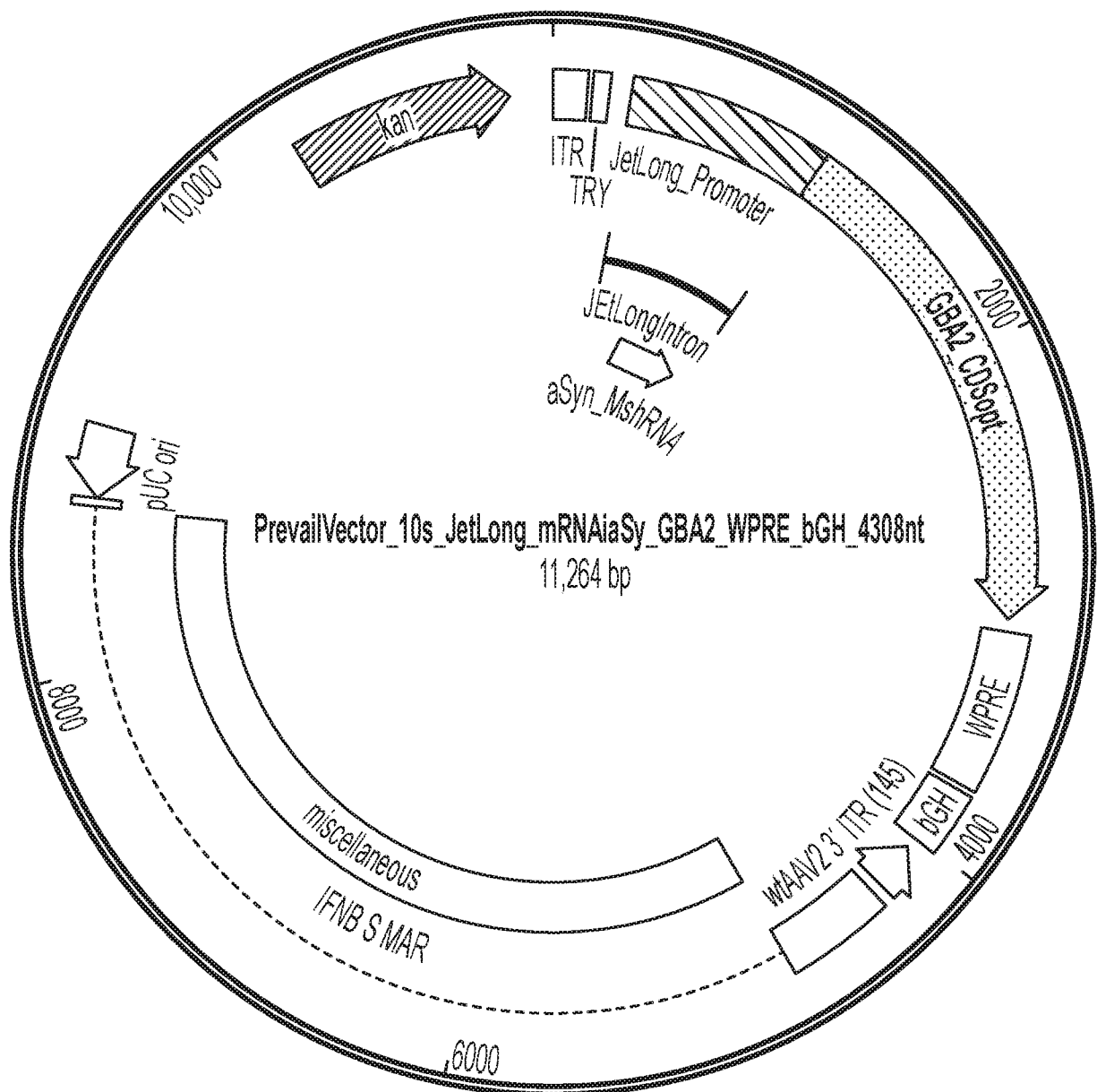


Figure 21

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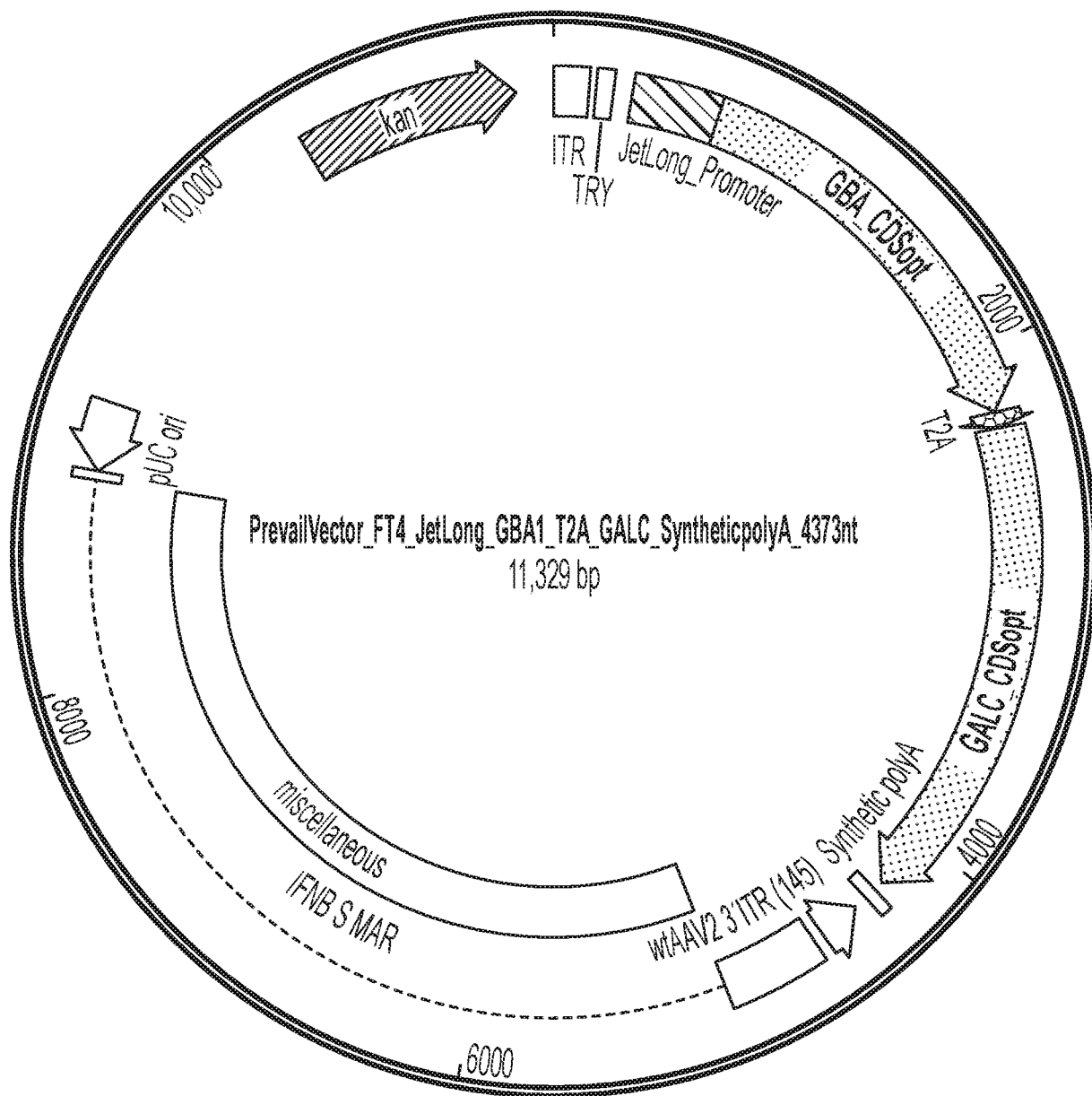


Figure 22

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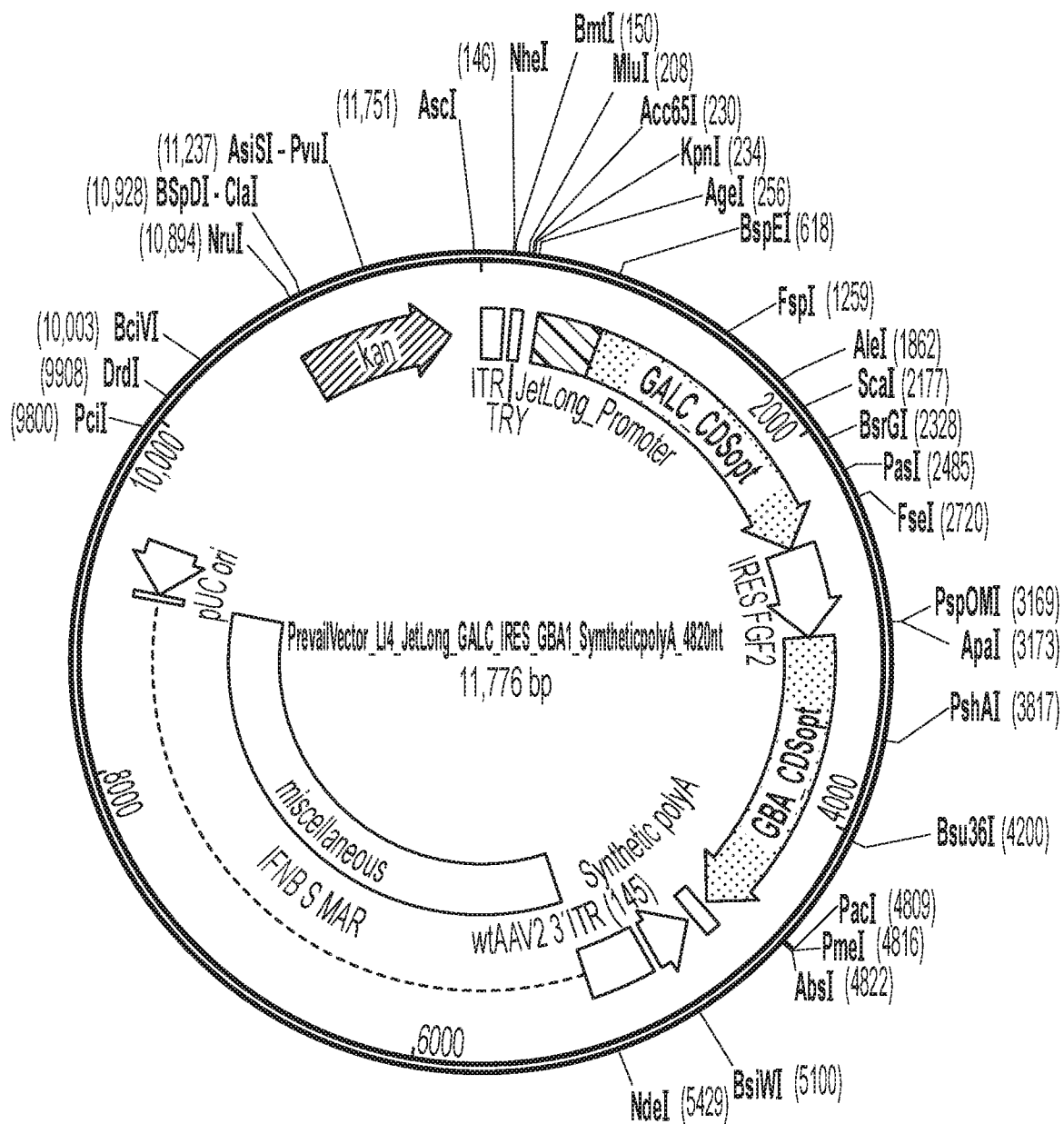


Figure 23

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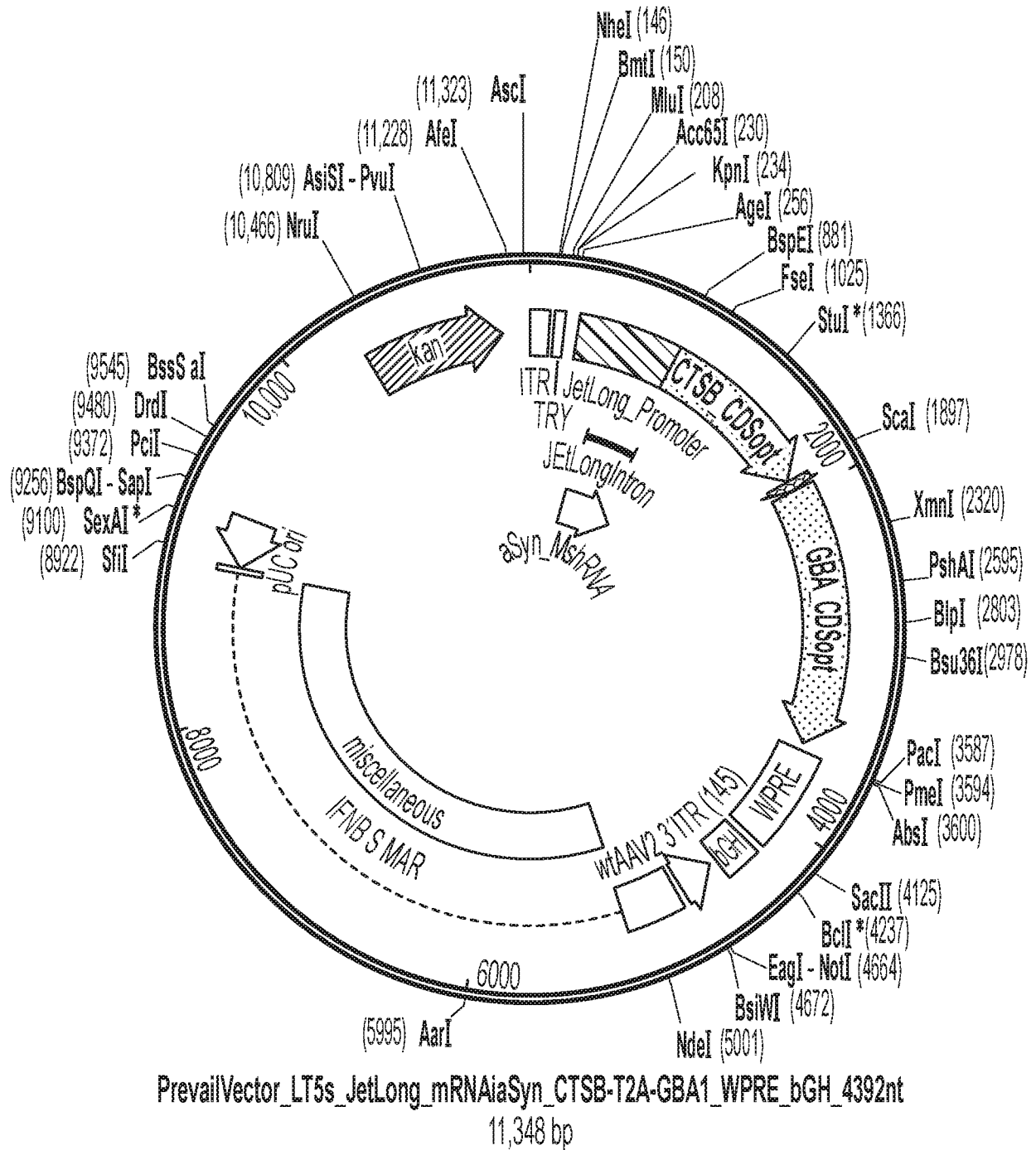


Figure 24

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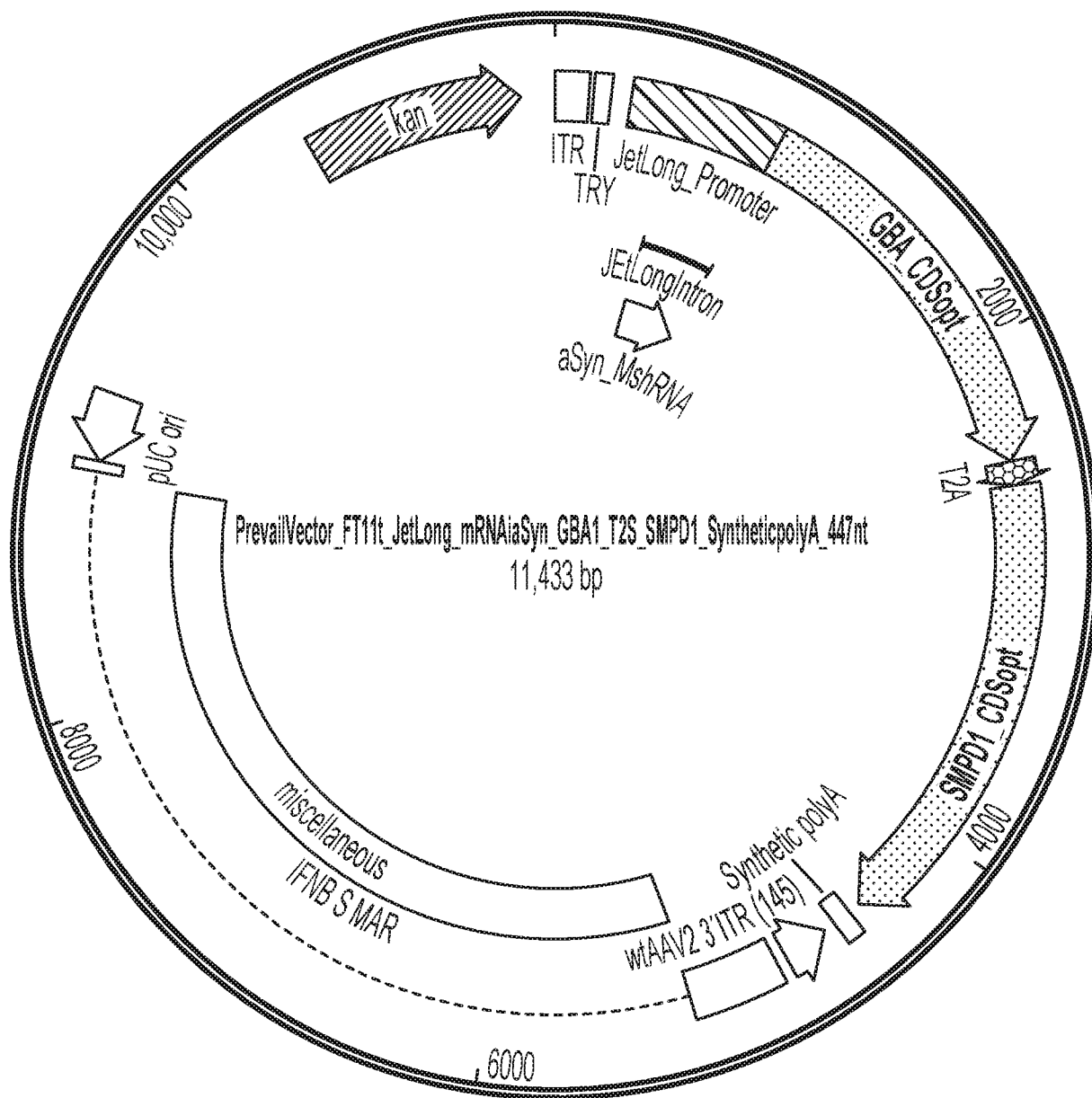


Figure 25

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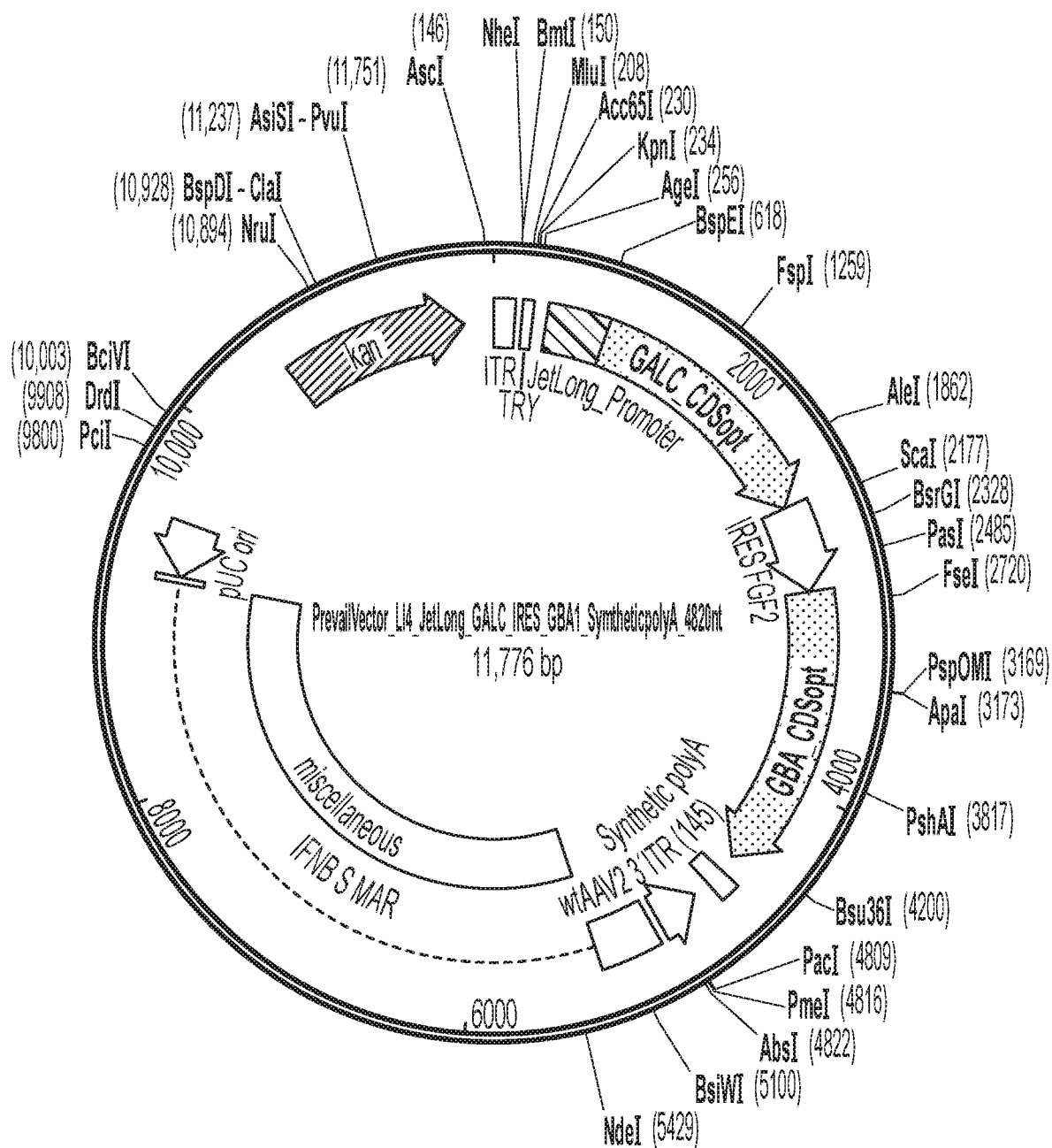


Figure 26

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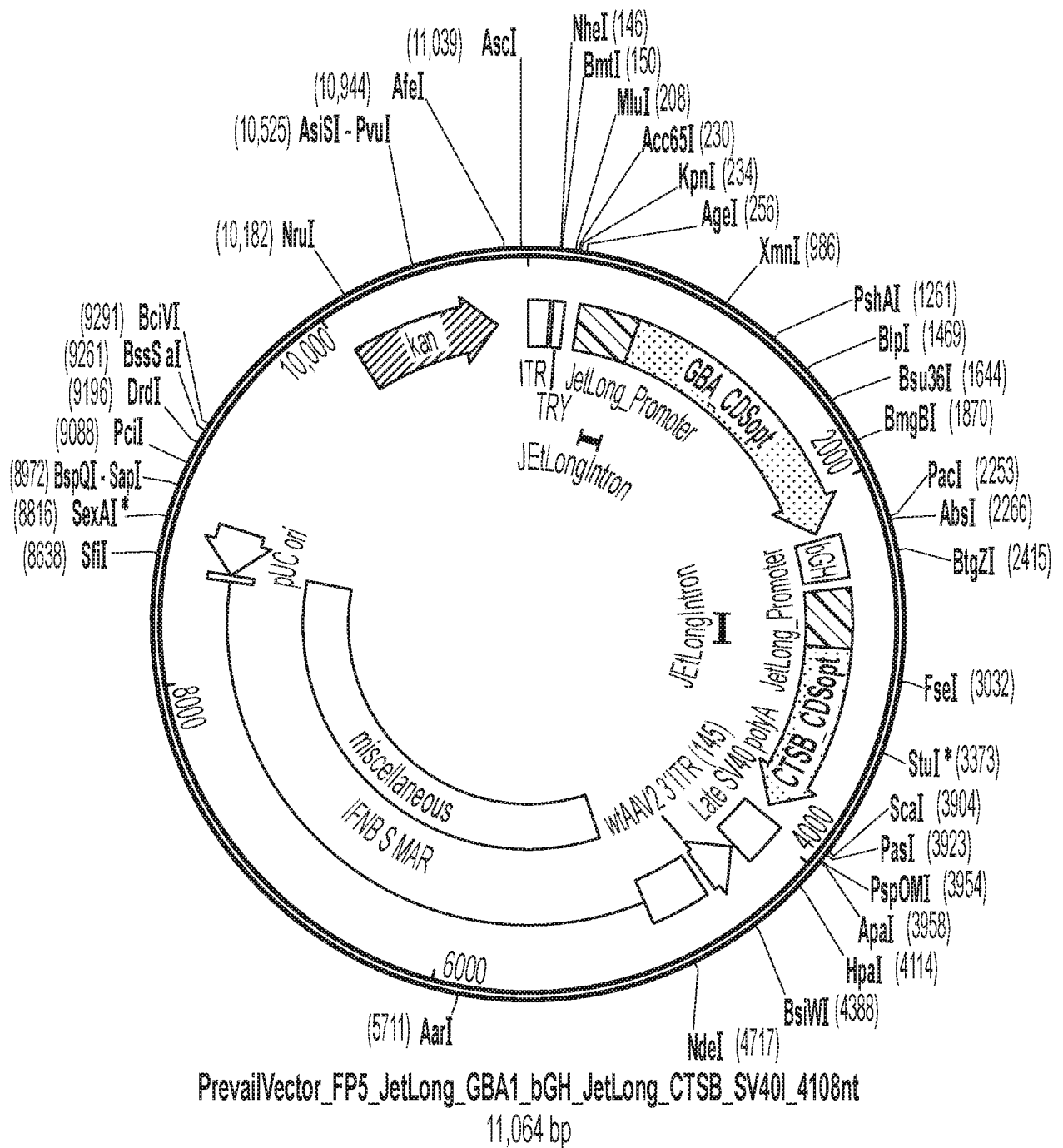


Figure 27

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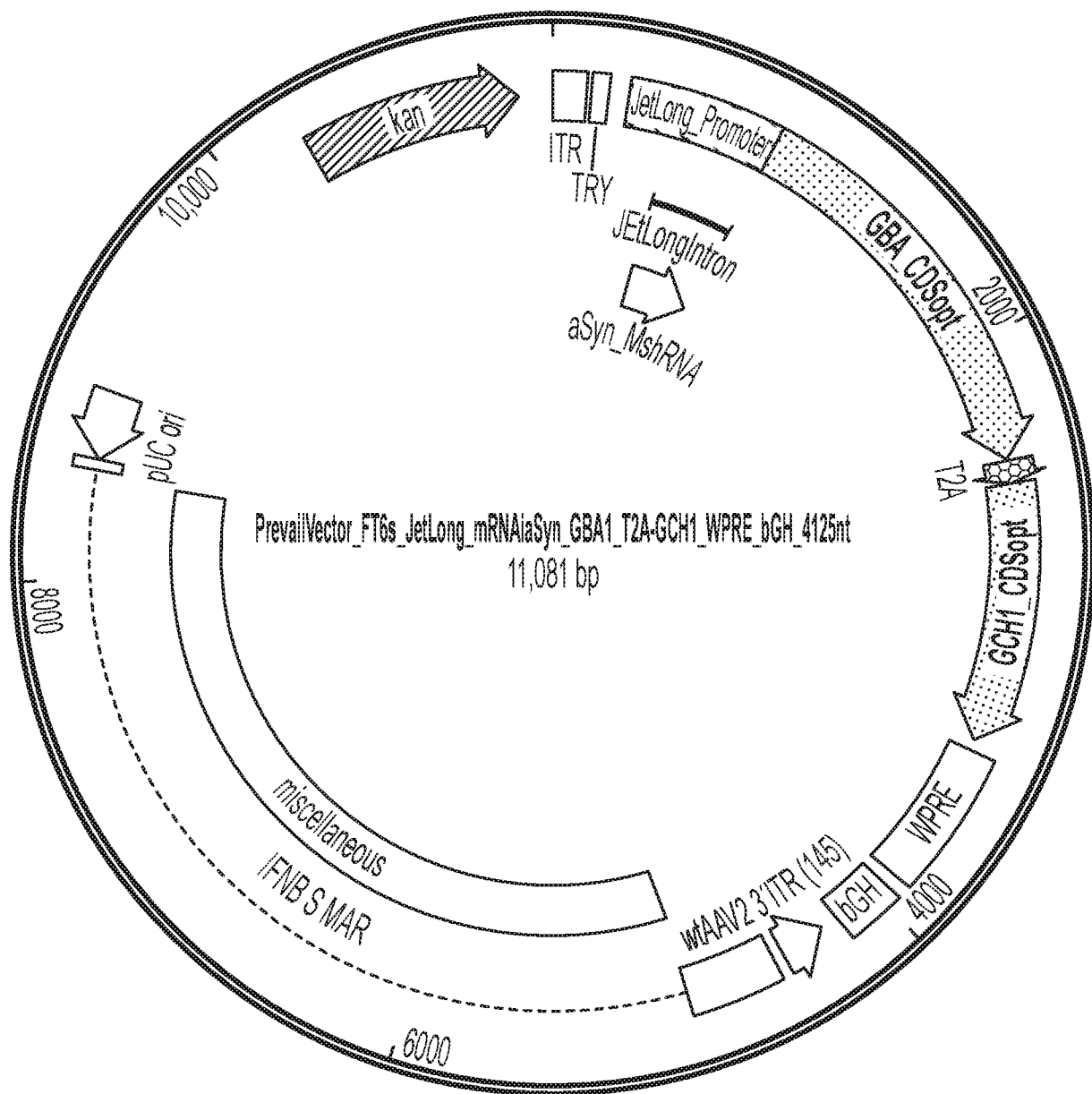


Figure 28

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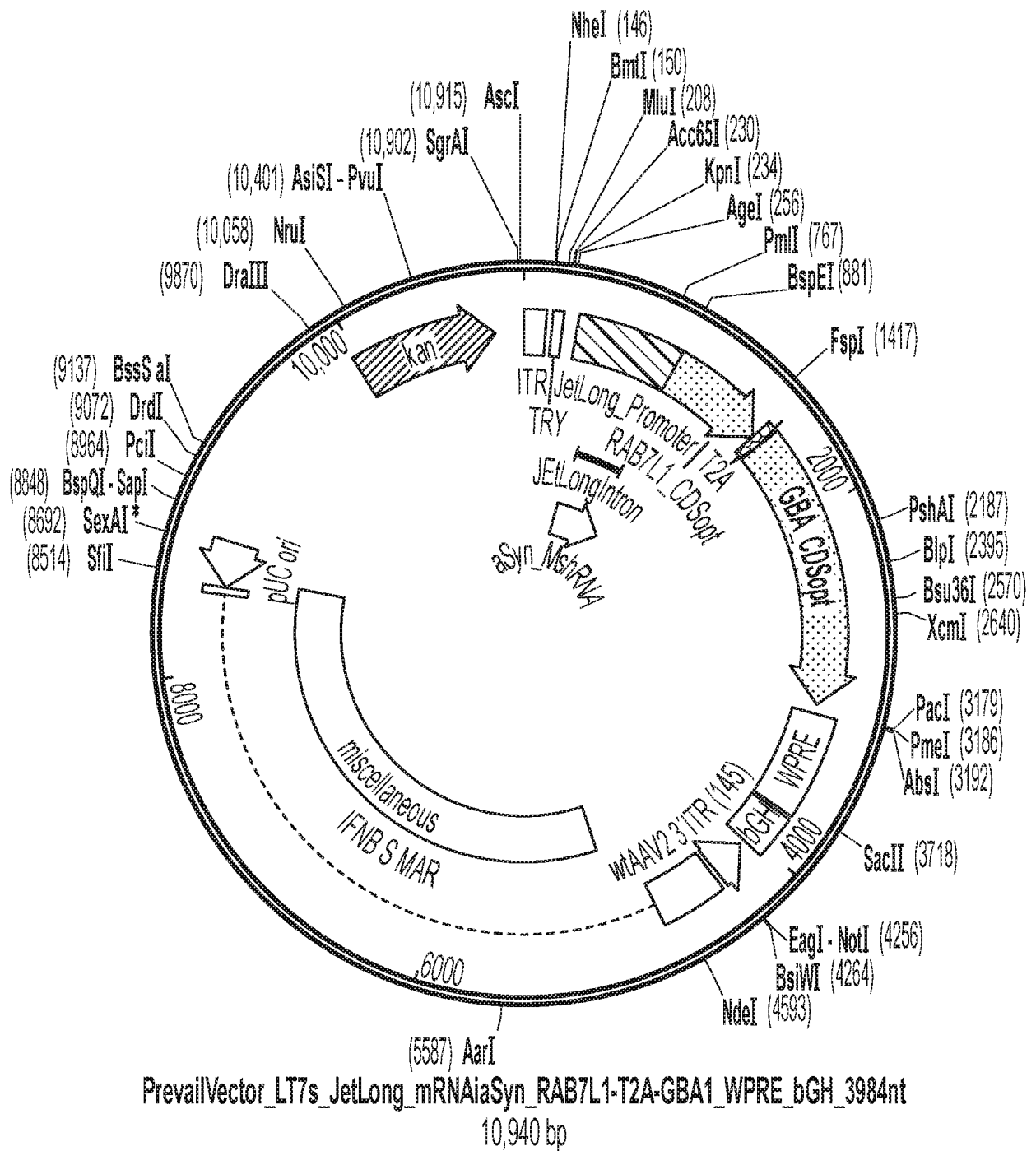


Figure 29

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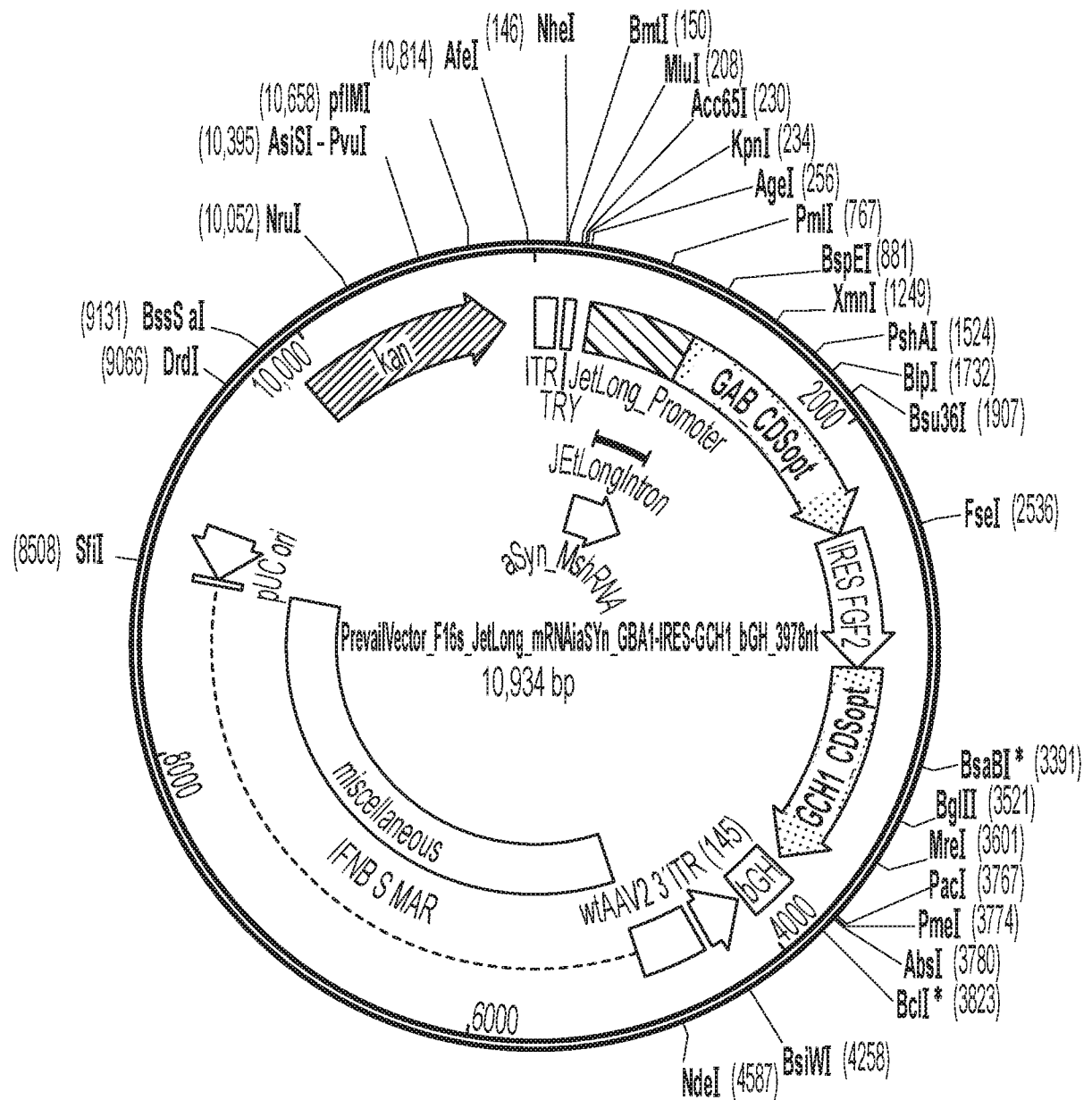


Figure 30

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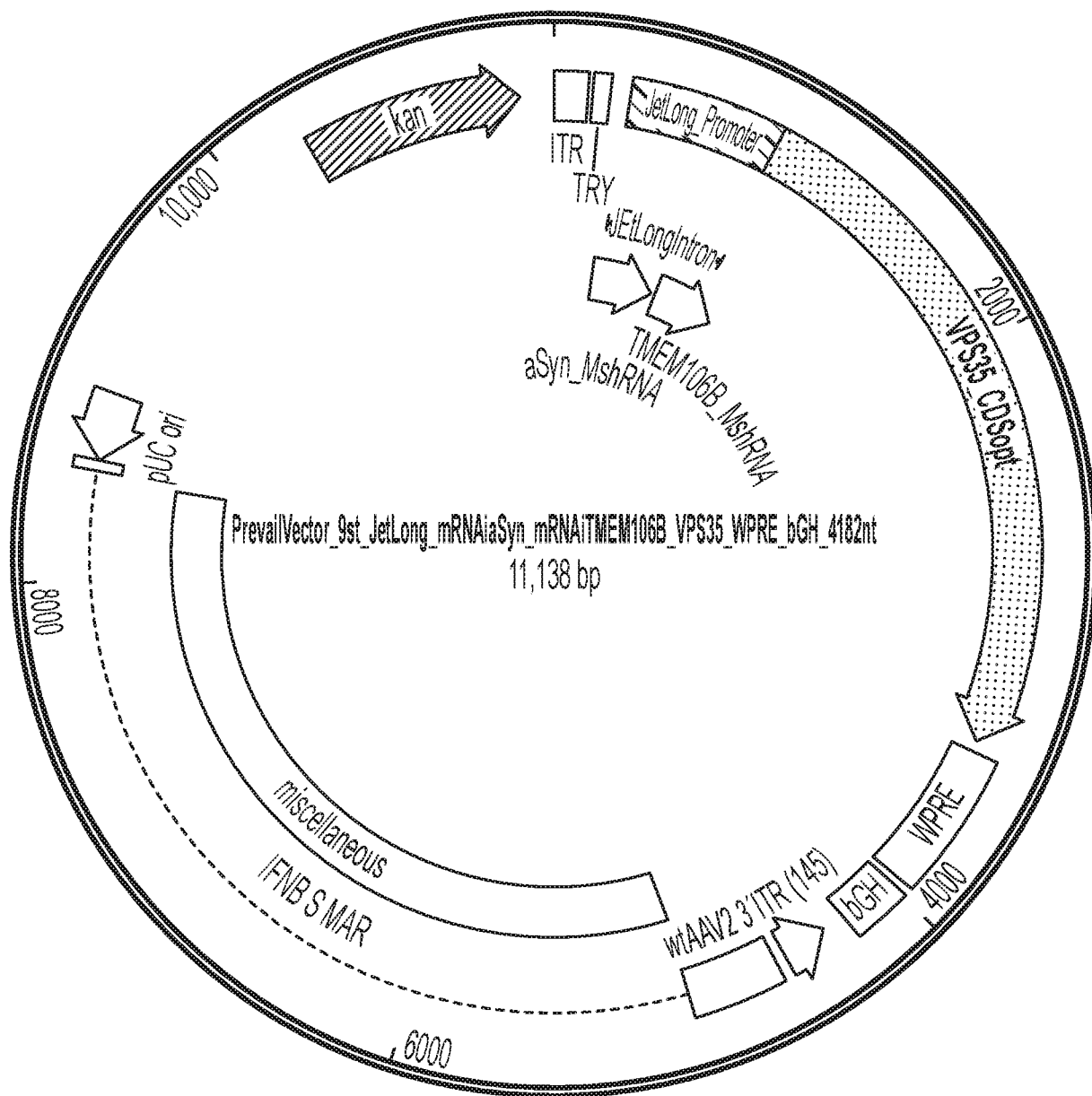


Figure 31

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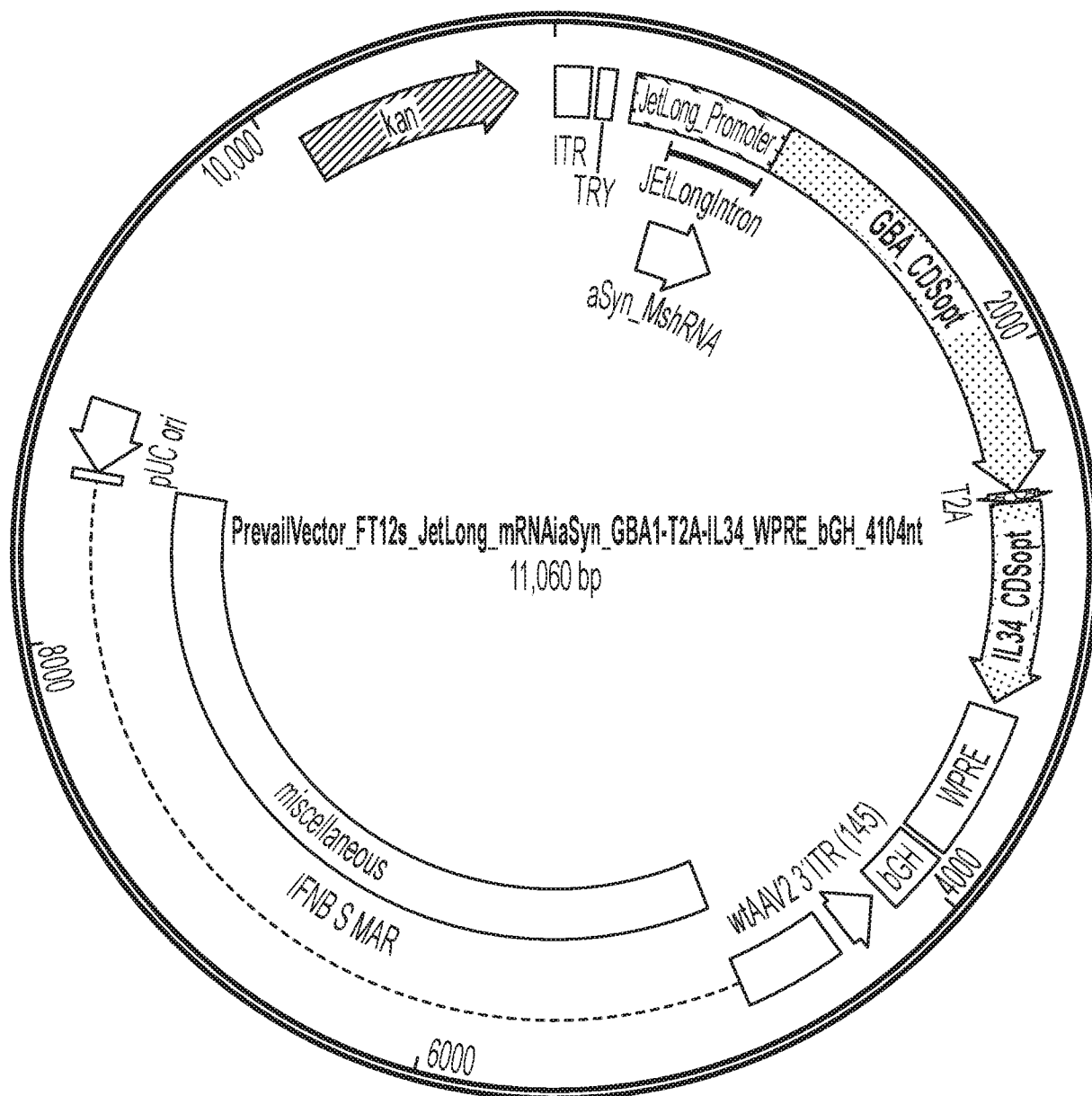
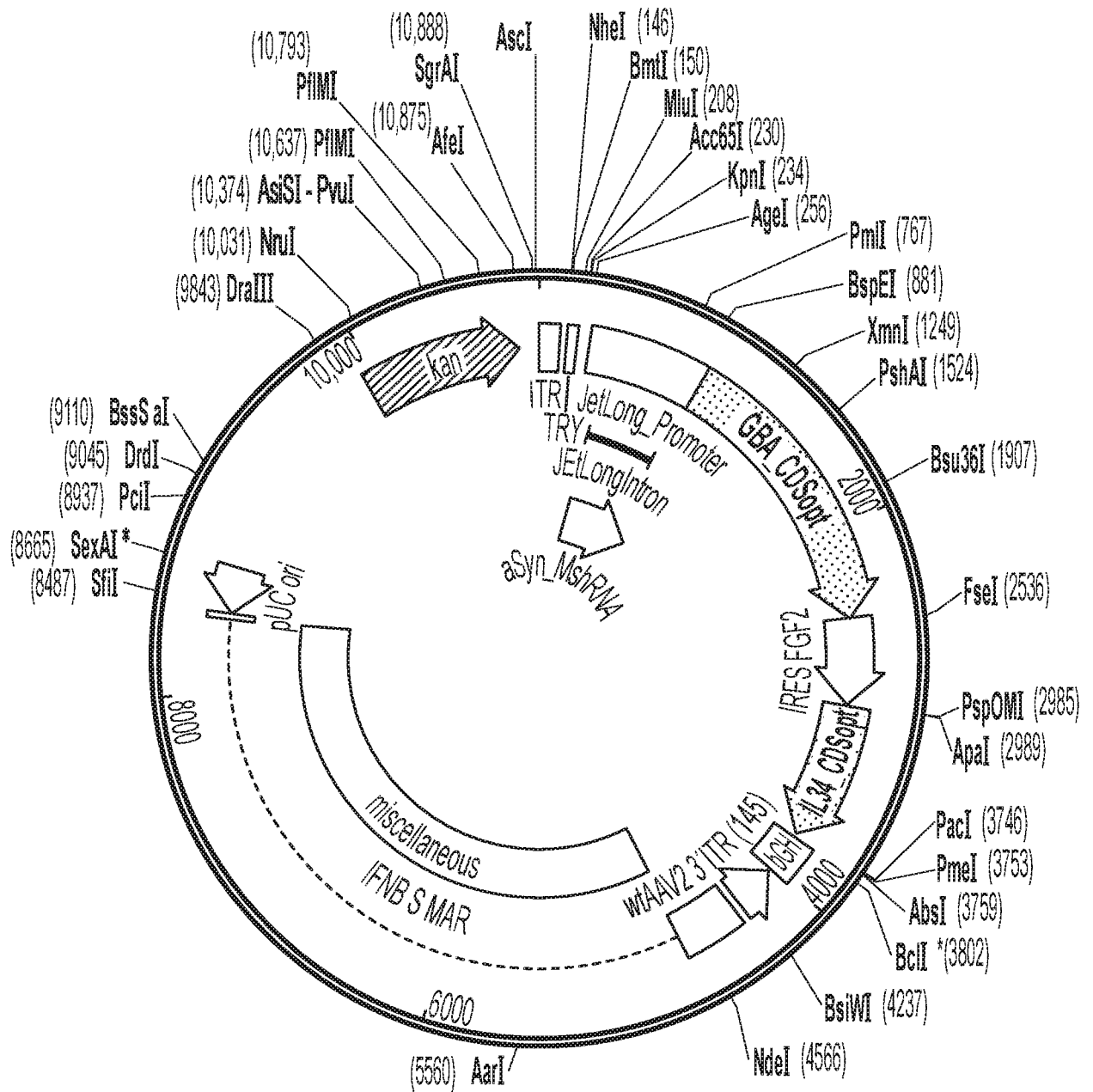


Figure 32



PrevailVector_F12s_JetLong_mRNAiaSYn_GBA1-IRES-IL34_bGH_3957nt

Figure 33

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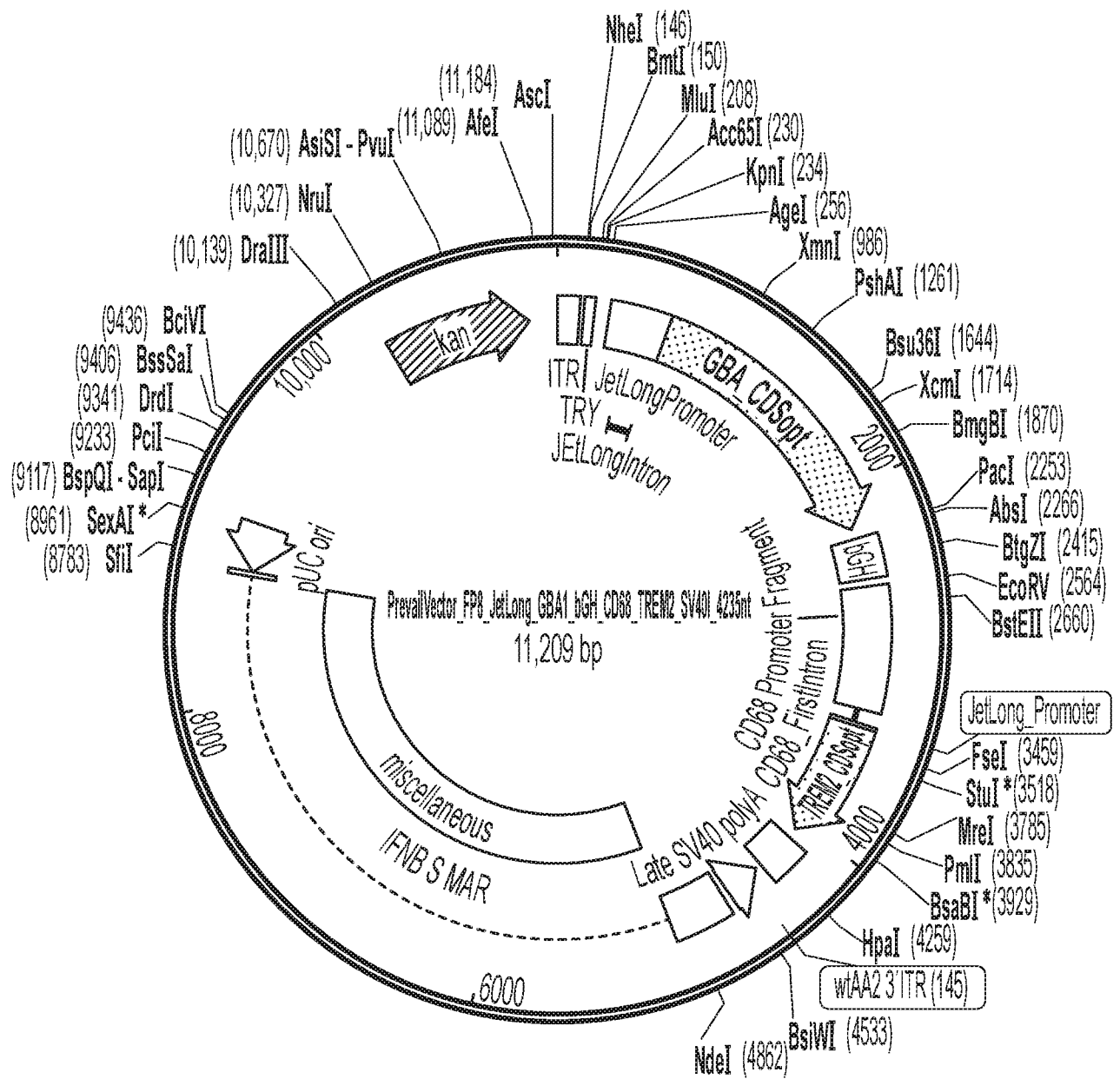
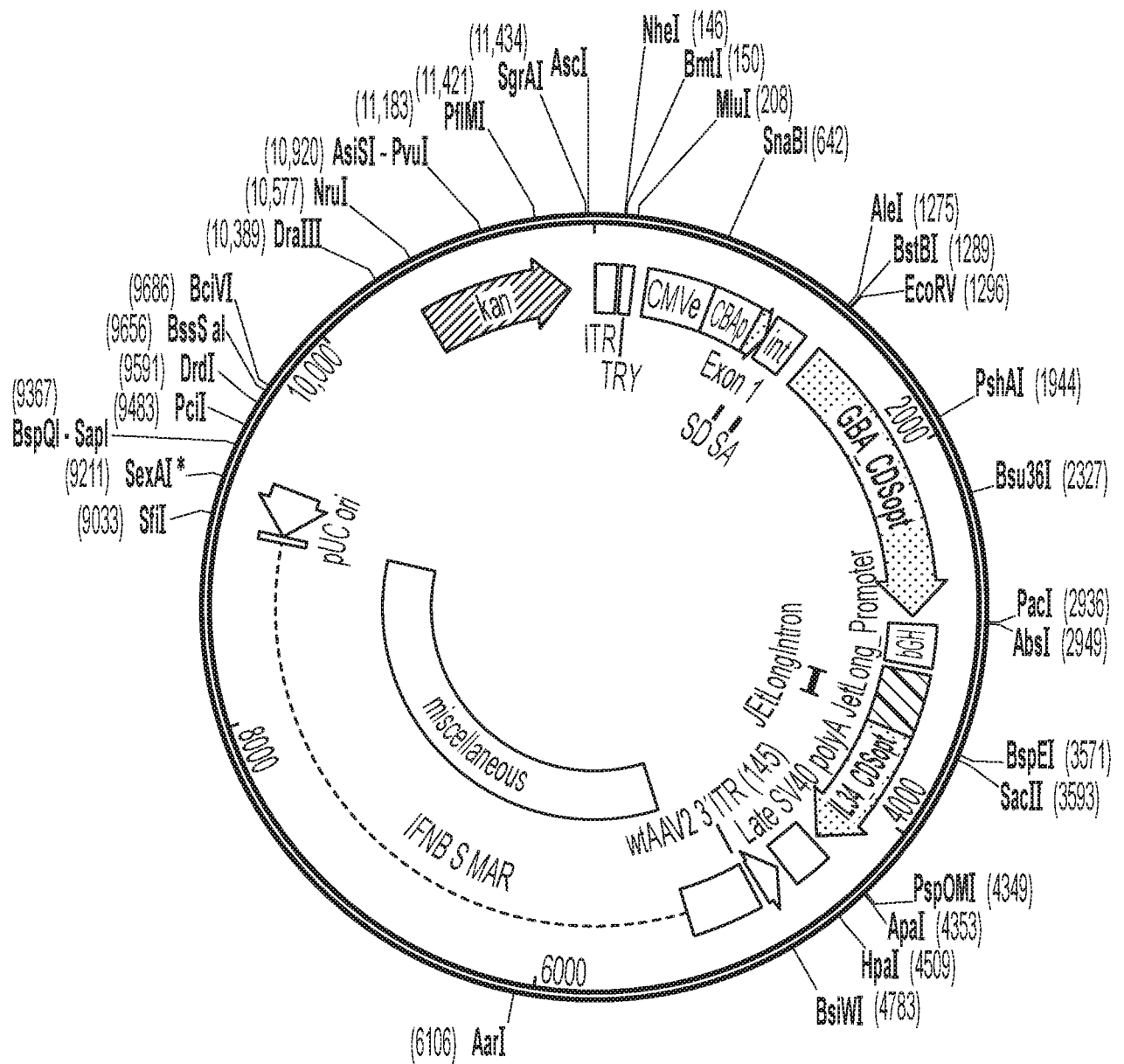


Figure 34



PrevailVector_FP12_CMVe_CBA_GBA1_bGH_JetLong_IL34_SV40I_4503nt

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Figure 35

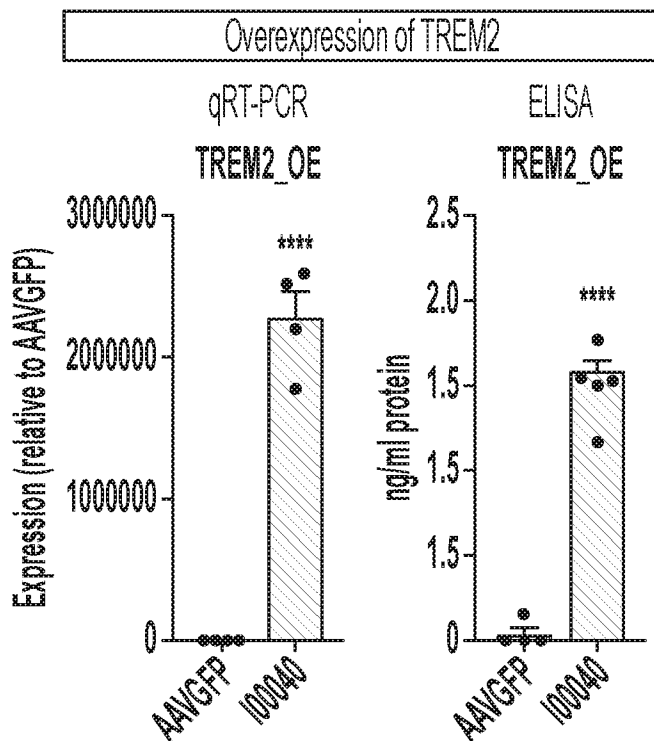


Figure 36A

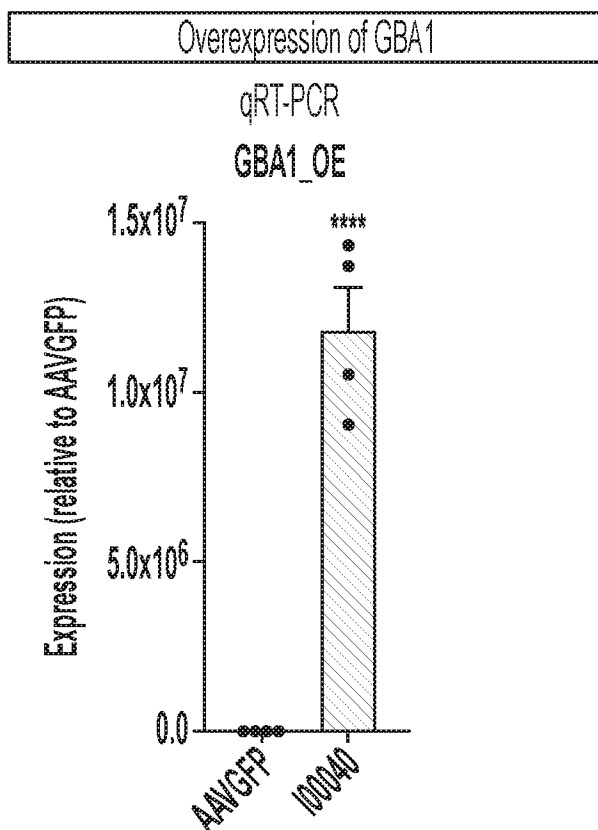


Figure 36B

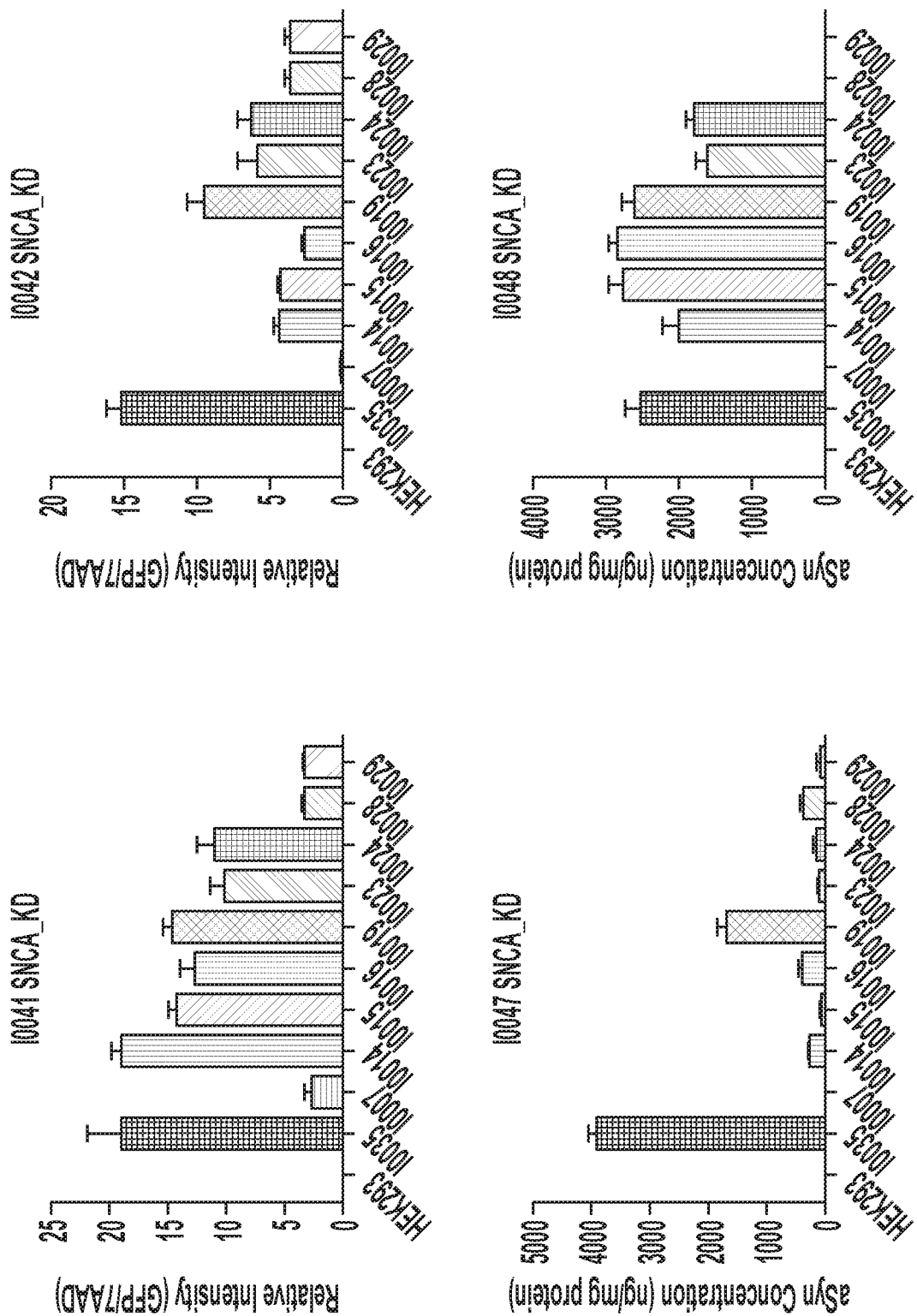


Figure 37

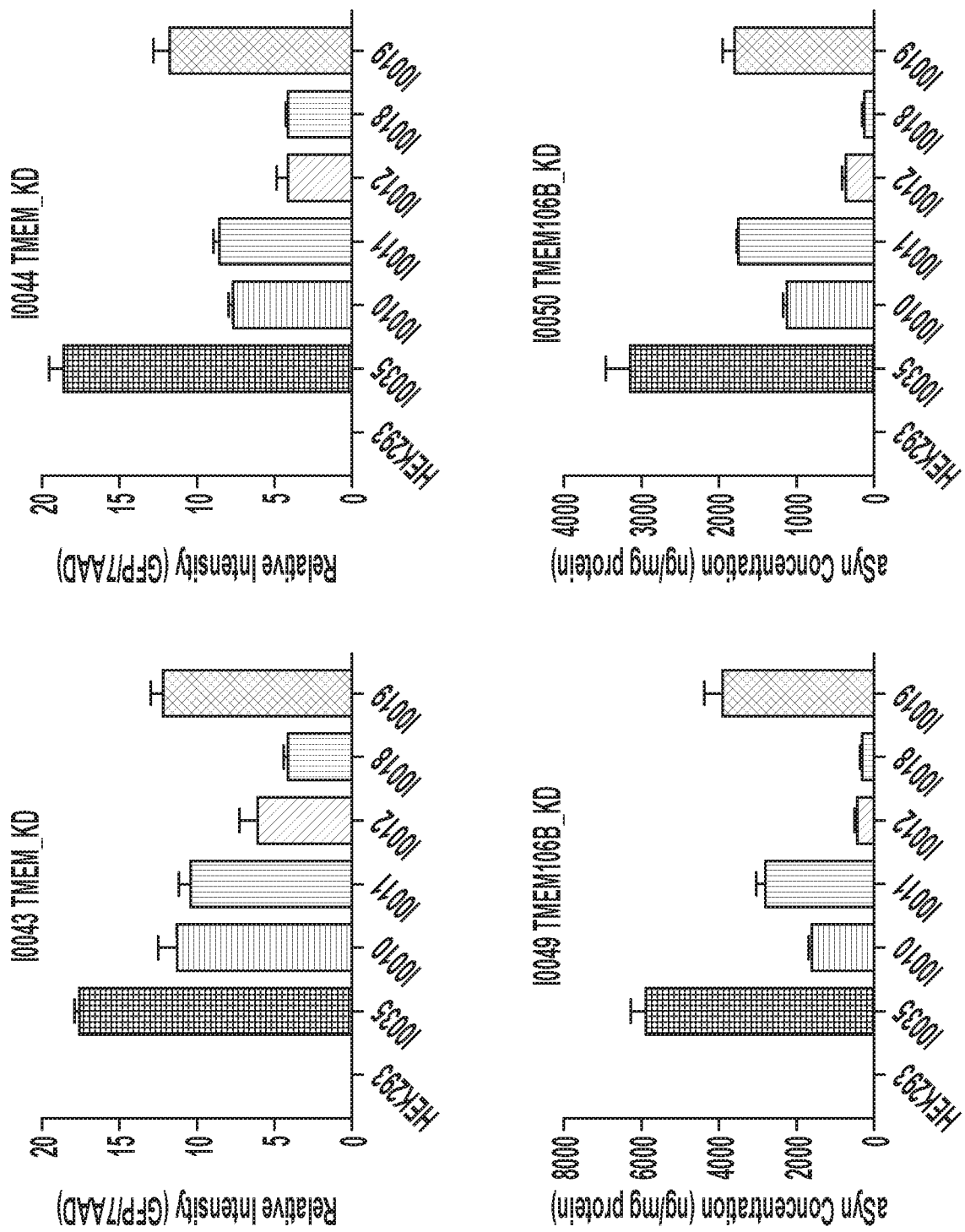


Figure 38

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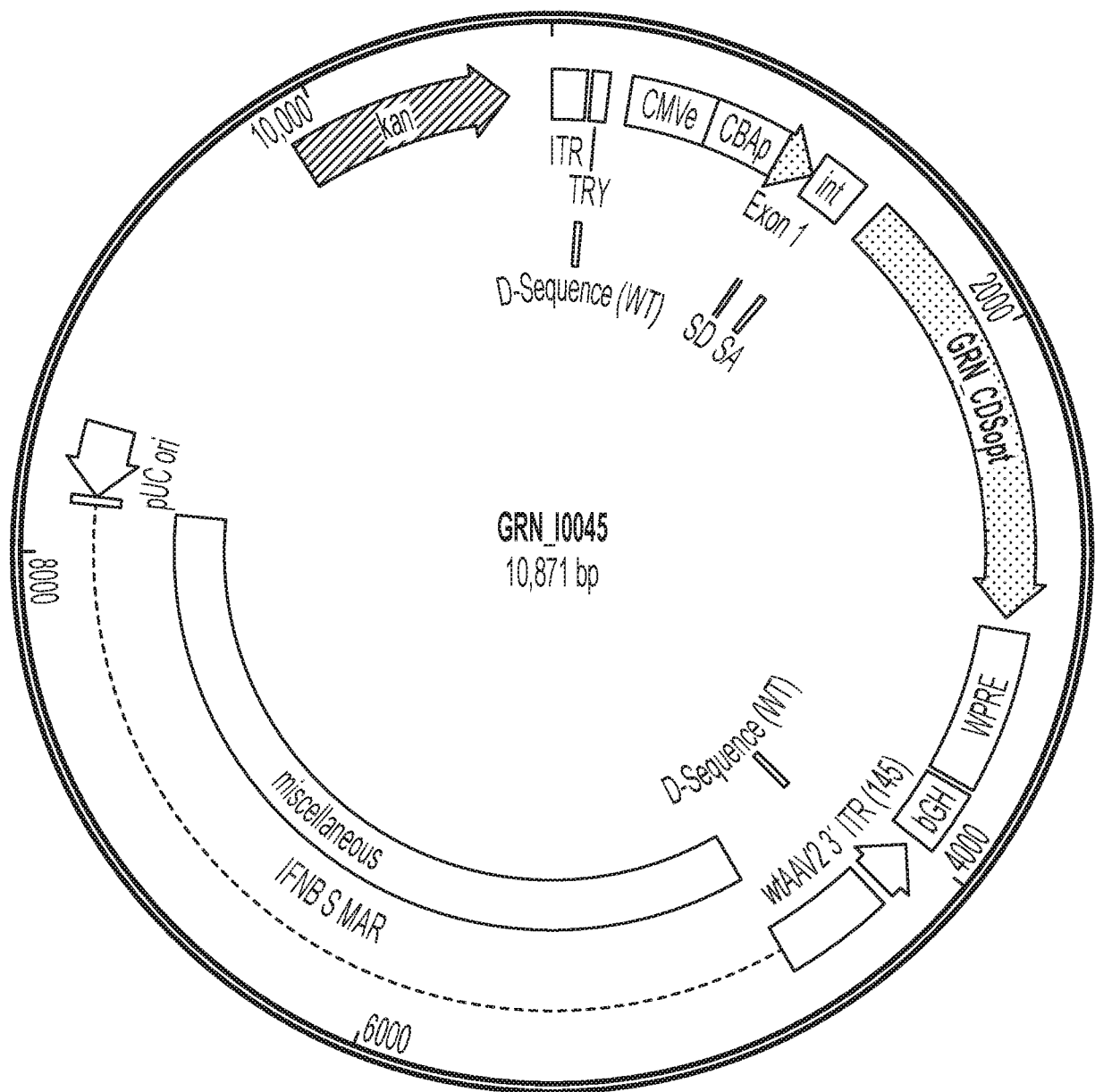


Figure 39

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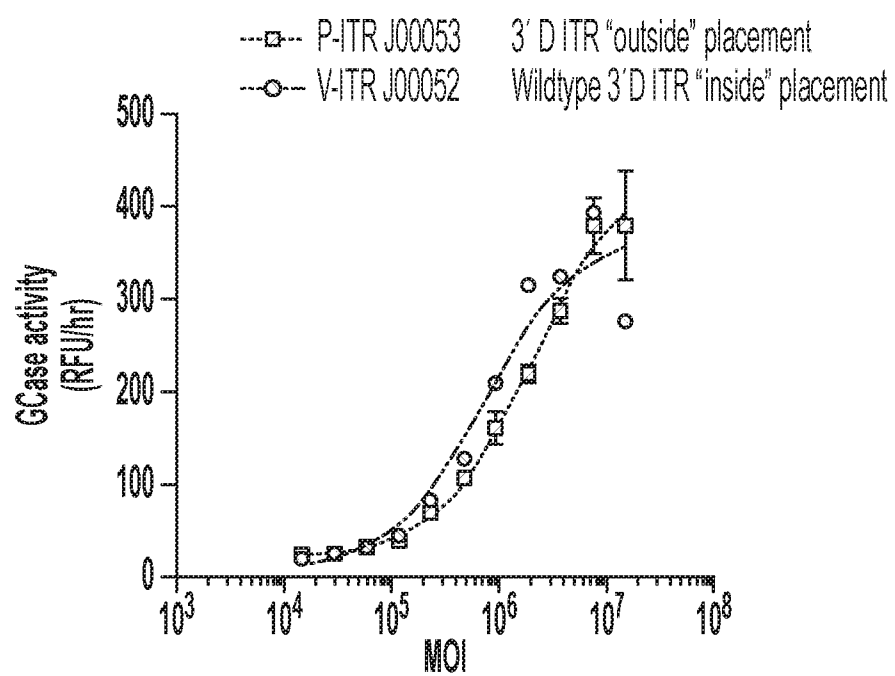


Figure 40

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aacctgctgc tcaagagcta cttcagcgag gaaggcatcg gctacaacat catcagagtg      480
cccatggcca gctgcgactt cagcatcagg acctacacct acgccgacac acccgacgat      540
ttccagctgc acaacttcag cctgcctgaa gaggacacca agctgaagat ccctctgatc      600
cacagagccc tgcagctggc acaaagacct gtgtcactgc tggcctctcc atggacatct      660
cccacctggc tgaaaacaaa tggcgccgtg aatggcaagg gcagcctgaa aggccaacct      720
ggcgacatct accaccagac ctgggccaga tacttcgtga agttcctgga cgcctatgcc      780
gagcacaagc tgagtttttg ggccgtgaca gccgagaacg aaccttctgc tggactgctg      840
agcgggtacc cttttcagtg cctgggcttt acacccgagc accagcggga ctttatcgcc      900
cgtgatctgg gaccacact ggccaatagc acccaccata atgtgctggc gctgatgctg      960

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gacgaccaga gactgcttct gccccactgg gctaaagtgg tgctgacaga tcctgaggcc 1020
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gccacactgg gagagacaca cagactgttc cccaacacca tgctgttcgc cagcgaagcc 1140
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cagtacagcc acagcatcat caccaacctg ctgtaccacg tcgtcggctg gaccgactgg 1260
aatctggccc tgaatcctga aggcggccct aactgggtcc gaaacttcgt ggacagcccc 1320
atcatcgtgg acatcaccaa ggacaccttc tacaagcagc ccatgttcta ccacctggga 1380
cacttcagca agttcatccc cgagggctct cagcgcgttg gactggtggc ttcccagaag 1440
aacgatctgg acgccgtggc tctgatgcac cctgatggat ctgctgtggt ggtggtcctg 1500
aaccgcagca gcaaagatgt gcccctgacc atcaaggatc ccgccgtggg attcctggaa 1560
acaatcagcc ctggctactc catccacacc tacctgtggc gtagacag 1608

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<210> 16
 <211> 524
 <212> PRT
 <213> Homo sapiens

<400> 16

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Met Tyr Ala Leu Phe Leu Leu Ala Ser Leu Leu Gly Ala Ala Leu Ala
1           5           10          15

```

```

Gly Pro Val Leu Gly Leu Lys Glu Cys Thr Arg Gly Ser Ala Val Trp
          20          25          30

```

```

Cys Gln Asn Val Lys Thr Ala Ser Asp Cys Gly Ala Val Lys His Cys
          35          40          45

```

```

Leu Gln Thr Val Trp Asn Lys Pro Thr Val Lys Ser Leu Pro Cys Asp
          50          55          60

```

```

Ile Cys Lys Asp Val Val Thr Ala Ala Gly Asp Met Leu Lys Asp Asn

```

65						70													75										80
Ala	Thr	Glu	Glu	Glu	Ile	Leu	Val	Tyr	Leu	Glu	Lys	Thr	Cys	Asp	Trp														
				85					90					95															
Leu	Pro	Lys	Pro	Asn	Met	Ser	Ala	Ser	Cys	Lys	Glu	Ile	Val	Asp	Ser														
			100					105					110																
Tyr	Leu	Pro	Val	Ile	Leu	Asp	Ile	Ile	Lys	Gly	Glu	Met	Ser	Arg	Pro														
		115					120					125																	
Gly	Glu	Val	Cys	Ser	Ala	Leu	Asn	Leu	Cys	Glu	Ser	Leu	Gln	Lys	His														
		130					135					140																	
Leu	Ala	Glu	Leu	Asn	His	Gln	Lys	Gln	Leu	Glu	Ser	Asn	Lys	Ile	Pro														
145					150					155					160														
Glu	Leu	Asp	Met	Thr	Glu	Val	Val	Ala	Pro	Phe	Met	Ala	Asn	Ile	Pro														
				165					170					175															
Leu	Leu	Leu	Tyr	Pro	Gln	Asp	Gly	Pro	Arg	Ser	Lys	Pro	Gln	Pro	Lys														
			180					185					190																
Asp	Asn	Gly	Asp	Val	Cys	Gln	Asp	Cys	Ile	Gln	Met	Val	Thr	Asp	Ile														
		195					200					205																	
Gln	Thr	Ala	Val	Arg	Thr	Asn	Ser	Thr	Phe	Val	Gln	Ala	Leu	Val	Glu														
		210					215					220																	
His	Val	Lys	Glu	Glu	Cys	Asp	Arg	Leu	Gly	Pro	Gly	Met	Ala	Asp	Ile														
225					230					235					240														
Cys	Lys	Asn	Tyr	Ile	Ser	Gln	Tyr	Ser	Glu	Ile	Ala	Ile	Gln	Met	Met														
				245					250					255															
Met	His	Met	Gln	Pro	Lys	Glu	Ile	Cys	Ala	Leu	Val	Gly	Phe	Cys	Asp														

260

265

270

Glu Val Lys Glu Met Pro Met Gln Thr Leu Val Pro Ala Lys Val Ala
 275 280 285

Ser Lys Asn Val Ile Pro Ala Leu Glu Leu Val Glu Pro Ile Lys Lys
 290 295 300

His Glu Val Pro Ala Lys Ser Asp Val Tyr Cys Glu Val Cys Glu Phe
 305 310 315 320

Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys
 325 330 335

Glu Ile Leu Asp Ala Phe Asp Lys Met Cys Ser Lys Leu Pro Lys Ser
 340 345 350

Leu Ser Glu Glu Cys Gln Glu Val Val Asp Thr Tyr Gly Ser Ser Ile
 355 360 365

Leu Ser Ile Leu Leu Glu Glu Val Ser Pro Glu Leu Val Cys Ser Met
 370 375 380

Leu His Leu Cys Ser Gly Thr Arg Leu Pro Ala Leu Thr Val His Val
 385 390 395 400

Thr Gln Pro Lys Asp Gly Gly Phe Cys Glu Val Cys Lys Lys Leu Val
 405 410 415

Gly Tyr Leu Asp Arg Asn Leu Glu Lys Asn Ser Thr Lys Gln Glu Ile
 420 425 430

Leu Ala Ala Leu Glu Lys Gly Cys Ser Phe Leu Pro Asp Pro Tyr Gln
 435 440 445

Lys Gln Cys Asp Gln Phe Val Ala Glu Tyr Glu Pro Val Leu Ile Glu

450

455

460

Ile Leu Val Glu Val Met Asp Pro Ser Phe Val Cys Leu Lys Ile Gly
 465 470 475 480

Ala Cys Pro Ser Ala His Lys Pro Leu Leu Gly Thr Glu Lys Cys Ile
 485 490 495

Trp Gly Pro Ser Tyr Trp Cys Gln Asn Thr Glu Thr Ala Ala Gln Cys
 500 505 510

Asn Ala Val Glu His Cys Lys Arg His Val Trp Asn
 515 520

<210> 17
 <211> 1572
 <212> DNA
 <213> Homo sapiens

<400> 17
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 gactgcggcg ccgtgaagca ctgcctgcag accgtgtgga acaagcccac cgtgaagagc 180
 ctgccctgcg acatctgcaa ggacgtggtg accgccgccg gcgacatgct gaaggacaac 240
 gccaccgagg aggagatcct ggtgtacctg gagaagacct gcgactggct gccaagccc 300
 aacatgagcg ccagctgcaa ggagatcgtg gacagctacc tgcccgtgat cctggacatc 360
 atcaagggcg agatgagccg ccccggcgag gtgtgcagcg ccctgaacct gtgcgagagc 420
 ctgcagaagc acctggccga gctgaaccac cagaagcagc tggagagcaa caagatcccc 480
 gagctggaca tgaccgaggt ggtggcccc ttcatggcca acatccccct gctgctgtac 540
 ccccgaggacg gccccgcag caagccccag cccaaggaca acggcgacgt gtgccaggac 600
 tgcacccaga tggtagccga catccagacc gccgtgcgca ccaacagcac cttcgtgcag 660
 gccctggtgg agcacgtgaa ggaggagtgc gaccgcctgg gccccggcat ggccgacatc 720

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tgcaagaact acatcagcca gtacagcgag atcgccatcc agatgatgat gcacatgcag      780
cccaaggaga tctgcgccct ggtgggcttc tgcgacgagg tgaaggagat gcccatgcag      840
accctggtgc ccgccaaggt ggccagcaag aacgtgatcc ccgccctgga gctggtggag      900
cccatcaaga agcacgaggt gcccgccaag agcgacgtgt actgcgaggt gtgcgagttc      960
ctggtgaagg aggtgaccaa gctgatcgac aacaacaaga ccgagaagga gatacctggac     1020
gccttcgaca agatgtgcag caagctgccc aagagcctga gcgaggagtg ccaggaggtg     1080
gtggacacct acggcagcag catcctgagc atcctgctgg aggaggtgag ccccgagctg     1140
gtgtgcagca tgctgcacct gtgcagcggc acccgctgc ccgccctgac cgtgcacgtg     1200
accagccca aggacggcgg cttctgcgag gtgtgcaaga agctggtggg ctacctggac     1260
cgcaacctgg agaagaacag caccaagcag gagatcctgg ccgccctgga gaagggtgc      1320
agcttcctgc ccgacccta ccagaagcag tgcgaccagt tcgtggccga gtacgagccc      1380
gtgctgatcg agatcctggt ggaggtgatg gacccagct tcgtgtgcct gaagatcggc      1440
gcctgcccca gcgcccacaa gcccctgctg ggcaccgaga agtgcattctg gggccccagc      1500
tactggtgcc agaacaccga gaccgccgcc cagtgcacg ccgtggagca ctgcaagcgc      1560
cacgtgtgga ac                                                    1572

```

```

<210> 18
<211> 478
<212> PRT
<213> Homo sapiens

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<400> 18
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Met Gly Arg Cys Cys Phe Tyr Thr Ala Gly Thr Leu Ser Leu Leu Leu
1           5           10           15

```

```

Leu Val Thr Ser Val Thr Leu Leu Val Ala Arg Val Phe Gln Lys Ala
          20           25           30

```

```
Val Asp Gln Ser Ile Glu Lys Lys Ile Val Leu Arg Asn Gly Thr Glu
```

35

40

45

Ala Phe Asp Ser Trp Glu Lys Pro Pro Leu Pro Val Tyr Thr Gln Phe
 50 55 60

Tyr Phe Phe Asn Val Thr Asn Pro Glu Glu Ile Leu Arg Gly Glu Thr
 65 70 75 80

Pro Arg Val Glu Glu Val Gly Pro Tyr Thr Tyr Arg Glu Leu Arg Asn
 85 90 95

Lys Ala Asn Ile Gln Phe Gly Asp Asn Gly Thr Thr Ile Ser Ala Val
 100 105 110

Ser Asn Lys Ala Tyr Val Phe Glu Arg Asp Gln Ser Val Gly Asp Pro
 115 120 125

Lys Ile Asp Leu Ile Arg Thr Leu Asn Ile Pro Val Leu Thr Val Ile
 130 135 140

Glu Trp Ser Gln Val His Phe Leu Arg Glu Ile Ile Glu Ala Met Leu
 145 150 155 160

Lys Ala Tyr Gln Gln Lys Leu Phe Val Thr His Thr Val Asp Glu Leu
 165 170 175

Leu Trp Gly Tyr Lys Asp Glu Ile Leu Ser Leu Ile His Val Phe Arg
 180 185 190

Pro Asp Ile Ser Pro Tyr Phe Gly Leu Phe Tyr Glu Lys Asn Gly Thr
 195 200 205

Asn Asp Gly Asp Tyr Val Phe Leu Thr Gly Glu Asp Ser Tyr Leu Asn
 210 215 220

Phe Thr Lys Ile Val Glu Trp Asn Gly Lys Thr Ser Leu Asp Trp Trp

225		230		235		240									
Ile	Thr	Asp	Lys	Cys	Asn	Met	Ile	Asn	Gly	Thr	Asp	Gly	Asp	Ser	Phe
				245					250					255	
His	Pro	Leu	Ile	Thr	Lys	Asp	Glu	Val	Leu	Tyr	Val	Phe	Pro	Ser	Asp
			260					265					270		
Phe	Cys	Arg	Ser	Val	Tyr	Ile	Thr	Phe	Ser	Asp	Tyr	Glu	Ser	Val	Gln
		275						280				285			
Gly	Leu	Pro	Ala	Phe	Arg	Tyr	Lys	Val	Pro	Ala	Glu	Ile	Leu	Ala	Asn
	290					295					300				
Thr	Ser	Asp	Asn	Ala	Gly	Phe	Cys	Ile	Pro	Glu	Gly	Asn	Cys	Leu	Gly
305					310					315					320
Ser	Gly	Val	Leu	Asn	Val	Ser	Ile	Cys	Lys	Asn	Gly	Ala	Pro	Ile	Ile
				325					330					335	
Met	Ser	Phe	Pro	His	Phe	Tyr	Gln	Ala	Asp	Glu	Arg	Phe	Val	Ser	Ala
			340					345					350		
Ile	Glu	Gly	Met	His	Pro	Asn	Gln	Glu	Asp	His	Glu	Thr	Phe	Val	Asp
		355						360				365			
Ile	Asn	Pro	Leu	Thr	Gly	Ile	Ile	Leu	Lys	Ala	Ala	Lys	Arg	Phe	Gln
	370					375					380				
Ile	Asn	Ile	Tyr	Val	Lys	Lys	Leu	Asp	Asp	Phe	Val	Glu	Thr	Gly	Asp
385					390					395					400
Ile	Arg	Thr	Met	Val	Phe	Pro	Val	Met	Tyr	Leu	Asn	Glu	Ser	Val	His
			405					410						415	
Ile	Asp	Lys	Glu	Thr	Ala	Ser	Arg	Leu	Lys	Ser	Met	Ile	Asn	Thr	Thr

420

425

430

Leu Ile Ile Thr Asn Ile Pro Tyr Ile Ile Met Ala Leu Gly Val Phe
 435 440 445

Phe Gly Leu Val Phe Thr Trp Leu Ala Cys Lys Gly Gln Gly Ser Met
 450 455 460

Asp Glu Gly Thr Ala Asp Glu Arg Ala Pro Leu Ile Arg Thr
 465 470 475

<210> 19
 <211> 1434
 <212> DNA
 <213> Homo sapiens

<400> 19
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 gtgaccctgc tgggtggcccg cgtgttccag aaggccgtgg accagagcat cgagaagaag 120
 atcgtgctgc gcaacggcac cgaggccttc gacagctggg agaagcccc cctgcccgtg 180
 tacaccagct tctacttctt caacgtgacc aaccccgagg agatcctgcg cggcgagacc 240
 ccccgcgctgg aggaggtggg cccctacacc taccgcgagc tgcgcaacaa ggccaacatc 300
 cagttcggcg acaacggcac caccatcagc gccgtgagca acaaggccta cgtgttcgag 360
 cgcgaccaga gcgtgggcga cccaagatc gacctgatcc gcaccctgaa catccccgtg 420
 ctgaccgtga tcgagtggag ccaggtgcac ttcctgcgcg agatcatcga ggccatgctg 480
 aaggcctacc agcagaagct gttcgtgacc cacaccgtgg acgagctgct gtggggctac 540
 aaggacgaga tcctgagcct gatccacgtg ttccgccccg acatcagccc ctacttcggc 600
 ctgttctacg agaagaacgg caccaacgac ggcgactacg tgttcctgac cggcgaggac 660
 agctacctga acttcaccaa gatcgtggag tggaacggca agaccagcct ggactggtgg 720
 atcaccgaca agtgcaacat gatcaacggc accgacggcg acagcttcca cccctgatc 780
 accaaggacg aggtgctgta cgtgttcccc agcgacttct gccgcagcgt gtacatcacc 840


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ttcagcgact acgagagcgt gcagggcctg cccgccttcc gctacaaggt gcccgccgag      900
atcctggcca acaccagcga caacgccggc ttctgcatcc ccgagggcaa ctgcctgggc      960
agcggcgtgc tgaacgtgag catctgcaag aacggcgccc ccatcatcat gagcttcccc     1020
cacttctacc aggccgacga gcgcttcgtg agcgccatcg agggcatgca cccaaccag      1080
gaggaccacg agaccttcgt ggacatcaac cccctgaccg gcatcatcct gaaggccgcc      1140
aagcgcttcc agatcaacat ctacgtgaag aagctggacg acttcgtgga gaccggcgac      1200
atccgcacca tgggtgttccc cgtgatgtac ctgaacgaga gcgtgcacat cgacaaggag      1260
accgccagcc gcctgaagag catgatcaac accaccctga tcatcaccaa catcccctac      1320
atcatcatgg ccctgggctgt gttcttcggc ctgggtgttca cctggctggc ctgcaagggc      1380
cagggcagca tggacgaggg caccgccgac gagcgcgccc ccctgatccg cacc           1434

```

```

<210> 20
<211> 23
<212> DNA
<213> Artificial sequence

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<220>
<223> Synthetic polynucleotide

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<400> 20
tggaagactt cgagatacac tgt                                           23

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```

<210> 21
<211> 23
<212> DNA
<213> Artificial sequence

```

```

<220>
<223> Synthetic polynucleotide

```

```

<400> 21
acagtgtatc tcgaagtctt cca                                           23

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```

<210> 22
<211> 21

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<212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 22
 tttagaaata agtggtagtc a 21

<210> 23
 <211> 21
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 23
 tgactaccac ttatttctaa a 21

<210> 24
 <211> 19
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 24
 agggatatcaa gactacgaa 19

<210> 25
 <211> 19
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 25
 ttcgtagtct tgataccct 19

<210> 26
 <211> 19

<212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 26
 tattagatct gatggccgc 19

 <210> 27
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 27
 ctccatcact aggggttcct 20

 <210> 28
 <211> 60
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic polynucleotide

 <400> 28
 agctctgggt atttaagccc gagtgagcac gcagggtctc cattttgaag cgggaggtta 60

 <210> 29
 <211> 145
 <212> DNA
 <213> Unknown

 <220>
 <223> AAV2 ITR

 <400> 29
 aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60

 ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagg 120

 gagcgcgcag agagggagtg gccaa 145

<210> 30
 <211> 927
 <212> PRT
 <213> Homo sapiens

<400> 30

Met Gly Thr Gln Asp Pro Gly Asn Met Gly Thr Gly Val Pro Ala Ser
 1 5 10 15

Glu Gln Ile Ser Cys Ala Lys Glu Asp Pro Gln Val Tyr Cys Pro Glu
 20 25 30

Glu Thr Gly Gly Thr Lys Asp Val Gln Val Thr Asp Cys Lys Ser Pro
 35 40 45

Glu Asp Ser Arg Pro Pro Lys Glu Thr Asp Cys Cys Asn Pro Glu Asp
 50 55 60

Ser Gly Gln Leu Met Val Ser Tyr Glu Gly Lys Ala Met Gly Tyr Gln
 65 70 75 80

Val Pro Pro Phe Gly Trp Arg Ile Cys Leu Ala His Glu Phe Thr Glu
 85 90 95

Lys Arg Lys Pro Phe Gln Ala Asn Asn Val Ser Leu Ser Asn Met Ile
 100 105 110

Lys His Ile Gly Met Gly Leu Arg Tyr Leu Gln Trp Trp Tyr Arg Lys
 115 120 125

Thr His Val Glu Lys Lys Thr Pro Phe Ile Asp Met Ile Asn Ser Val
 130 135 140

Pro Leu Arg Gln Ile Tyr Gly Cys Pro Leu Gly Gly Ile Gly Gly Gly
 145 150 155 160

P109470003W000-SEQ-KZM.txt

Thr Ile Thr Arg Gly Trp Arg Gly Gln Phe Cys Arg Trp Gln Leu Asn
165 170 175

Pro Gly Met Tyr Gln His Arg Thr Val Ile Ala Asp Gln Phe Thr Val
180 185 190

Cys Leu Arg Arg Glu Gly Gln Thr Val Tyr Gln Gln Val Leu Ser Leu
195 200 205

Glu Arg Pro Ser Val Leu Arg Ser Trp Asn Trp Gly Leu Cys Gly Tyr
210 215 220

Phe Ala Phe Tyr His Ala Leu Tyr Pro Arg Ala Trp Thr Val Tyr Gln
225 230 235 240

Leu Pro Gly Gln Asn Val Thr Leu Thr Cys Arg Gln Ile Thr Pro Ile
245 250 255

Leu Pro His Asp Tyr Gln Asp Ser Ser Leu Pro Val Gly Val Phe Val
260 265 270

Trp Asp Val Glu Asn Glu Gly Asp Glu Ala Leu Asp Val Ser Ile Met
275 280 285

Phe Ser Met Arg Asn Gly Leu Gly Gly Gly Asp Asp Ala Pro Gly Gly
290 295 300

Leu Trp Asn Glu Pro Phe Cys Leu Glu Arg Ser Gly Glu Thr Val Arg
305 310 315 320

Gly Leu Leu Leu His His Pro Thr Leu Pro Asn Pro Tyr Thr Met Ala
325 330 335

Val Ala Ala Arg Val Thr Ala Ala Thr Thr Val Thr His Ile Thr Ala
340 345 350

P109470003W000-SEQ-KZM.txt

Phe Asp Pro Asp Ser Thr Gly Gln Gln Val Trp Gln Asp Leu Leu Gln
355 360 365

Asp Gly Gln Leu Asp Ser Pro Thr Gly Gln Ser Thr Pro Thr Gln Lys
370 375 380

Gly Val Gly Ile Ala Gly Ala Val Cys Val Ser Ser Lys Leu Arg Pro
385 390 395 400

Arg Gly Gln Cys Arg Leu Glu Phe Ser Leu Ala Trp Asp Met Pro Arg
405 410 415

Ile Met Phe Gly Ala Lys Gly Gln Val His Tyr Arg Arg Tyr Thr Arg
420 425 430

Phe Phe Gly Gln Asp Gly Asp Ala Ala Pro Ala Leu Ser His Tyr Ala
435 440 445

Leu Cys Arg Tyr Ala Glu Trp Glu Glu Arg Ile Ser Ala Trp Gln Ser
450 455 460

Pro Val Leu Asp Asp Arg Ser Leu Pro Ala Trp Tyr Lys Ser Ala Leu
465 470 475 480

Phe Asn Glu Leu Tyr Phe Leu Ala Asp Gly Gly Thr Val Trp Leu Glu
485 490 495

Val Leu Glu Asp Ser Leu Pro Glu Glu Leu Gly Arg Asn Met Cys His
500 505 510

Leu Arg Pro Thr Leu Arg Asp Tyr Gly Arg Phe Gly Tyr Leu Glu Gly
515 520 525

Gln Glu Tyr Arg Met Tyr Asn Thr Tyr Asp Val His Phe Tyr Ala Ser
530 535 540

P109470003W000-SEQ-KZM.txt

Phe Ala Leu Ile Met Leu Trp Pro Lys Leu Glu Leu Ser Leu Gln Tyr
545 550 555 560

Asp Met Ala Leu Ala Thr Leu Arg Glu Asp Leu Thr Arg Arg Arg Tyr
565 570 575

Leu Met Ser Gly Val Met Ala Pro Val Lys Arg Arg Asn Val Ile Pro
580 585 590

His Asp Ile Gly Asp Pro Asp Asp Glu Pro Trp Leu Arg Val Asn Ala
595 600 605

Tyr Leu Ile His Asp Thr Ala Asp Trp Lys Asp Leu Asn Leu Lys Phe
610 615 620

Val Leu Gln Val Tyr Arg Asp Tyr Tyr Leu Thr Gly Asp Gln Asn Phe
625 630 635 640

Leu Lys Asp Met Trp Pro Val Cys Leu Ala Val Met Glu Ser Glu Met
645 650 655

Lys Phe Asp Lys Asp His Asp Gly Leu Ile Glu Asn Gly Gly Tyr Ala
660 665 670

Asp Gln Thr Tyr Asp Gly Trp Val Thr Thr Gly Pro Ser Ala Tyr Cys
675 680 685

Gly Gly Leu Trp Leu Ala Ala Val Ala Val Met Val Gln Met Ala Ala
690 695 700

Leu Cys Gly Ala Gln Asp Ile Gln Asp Lys Phe Ser Ser Ile Leu Ser
705 710 715 720

Arg Gly Gln Glu Ala Tyr Glu Arg Leu Leu Trp Asn Gly Arg Tyr Tyr
725 730 735

P109470003W000-SEQ-KZM.txt

Asn Tyr Asp Ser Ser Ser Arg Pro Gln Ser Arg Ser Val Met Ser Asp
740 745 750

Gln Cys Ala Gly Gln Trp Phe Leu Lys Ala Cys Gly Leu Gly Glu Gly
755 760 765

Asp Thr Glu Val Phe Pro Thr Gln His Val Val Arg Ala Leu Gln Thr
770 775 780

Ile Phe Glu Leu Asn Val Gln Ala Phe Ala Gly Gly Ala Met Gly Ala
785 790 795 800

Val Asn Gly Met Gln Pro His Gly Val Pro Asp Lys Ser Ser Val Gln
805 810 815

Ser Asp Glu Val Trp Val Gly Val Val Tyr Gly Leu Ala Ala Thr Met
820 825 830

Ile Gln Glu Gly Leu Thr Trp Glu Gly Phe Gln Thr Ala Glu Gly Cys
835 840 845

Tyr Arg Thr Val Trp Glu Arg Leu Gly Leu Ala Phe Gln Thr Pro Glu
850 855 860

Ala Tyr Cys Gln Gln Arg Val Phe Arg Ser Leu Ala Tyr Met Arg Pro
865 870 875 880

Leu Ser Ile Trp Ala Met Gln Leu Ala Leu Gln Gln Gln Gln His Lys
885 890 895

Lys Ala Ser Trp Pro Lys Val Lys Gln Gly Thr Gly Leu Arg Thr Gly
900 905 910

Pro Met Phe Gly Pro Lys Glu Ala Met Ala Asn Leu Ser Pro Glu
915 920 925

<210> 31
 <211> 2781
 <212> DNA
 <213> Homo sapiens

<400> 31
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 tgcgccaagg aggaccccca ggtgtactgc cccgaggaga ccggcggcac caaggacgtg 120
 caggtgaccg actgcaagag ccccaggagc agccgcccc ccaaggagac cgactgctgc 180
 aaccccgagg acagcggcca gctgatggtg agctacgagg gcaaggccat gggctaccag 240
 gtgccccct tcggctggcg catctgcctg gccacgagt tcaccgagaa gcgcaagccc 300
 ttccaggcca acaacgtgag cctgagcaac atgatcaagc acatcggcat gggcctgcgc 360
 tacctgcagt ggtggtaccg caagaccac gtggagaaga agaccctt catcgacatg 420
 atcaacagcg tgcccctgcg ccagatctac ggctgcccc tgggcggcat cggcggcggc 480
 accatcacc gcggctggcg cggccagttc tgccgctggc agctgaacc cggcatgtac 540
 cagcaccgca ccgtgatcgc cgaccagttc accgtgtgcc tgcgccgca gggccagacc 600
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gccatcctat ggaactgcct cggtaggttt tctccttcat tacagaaacg gctttttcaa	11040
aaatatggta ttgataatcc tgatatgaat aaattgcagt ttcatttgat gctcgatgag	11100

tttttctaag ggcggcctgc caccataccc acgccgaaac aagcgctcat gagcccgaag 11160
 tggcgagccc gatcttcccc atcgggtgatg tcggcgatat aggcgccagc aaccgcacct 11220
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<210> 33
 <211> 685
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic polypeptide

<400> 33

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Met Thr Ala Ala Ala Gly Ser Ala Gly Arg Ala Ala Val Pro Leu Leu
 20 25 30

Leu Cys Ala Leu Leu Ala Pro Gly Gly Ala Tyr Val Leu Asp Asp Ser
 35 40 45

Asp Gly Leu Gly Arg Glu Phe Asp Gly Ile Gly Ala Val Ser Gly Gly
 50 55 60

Gly Ala Thr Ser Arg Leu Leu Val Asn Tyr Pro Glu Pro Tyr Arg Ser
 65 70 75 80

Gln Ile Leu Asp Tyr Leu Phe Lys Pro Asn Phe Gly Ala Ser Leu His
 85 90 95

Ile Leu Lys Val Glu Ile Gly Gly Asp Gly Gln Thr Thr Asp Gly Thr
 100 105 110

Glu Pro Ser His Met His Tyr Ala Leu Asp Glu Asn Tyr Phe Arg Gly
 115 120 125

Tyr Glu Trp Trp Leu Met Lys Glu Ala Lys Lys Arg Asn Pro Asn Ile
130 135 140

Thr Leu Ile Gly Leu Pro Trp Ser Phe Pro Gly Trp Leu Gly Lys Gly
145 150 155 160

Phe Asp Trp Pro Tyr Val Asn Leu Gln Leu Thr Ala Tyr Tyr Val Val
165 170 175

Thr Trp Ile Val Gly Ala Lys Arg Tyr His Asp Leu Asp Ile Asp Tyr
180 185 190

Ile Gly Ile Trp Asn Glu Arg Ser Tyr Asn Ala Asn Tyr Ile Lys Ile
195 200 205

Leu Arg Lys Met Leu Asn Tyr Gln Gly Leu Gln Arg Val Lys Ile Ile
210 215 220

Ala Ser Asp Asn Leu Trp Glu Ser Ile Ser Ala Ser Met Leu Leu Asp
225 230 235 240

Ala Glu Leu Phe Lys Val Val Asp Val Ile Gly Ala His Tyr Pro Gly
245 250 255

Thr His Ser Ala Lys Asp Ala Lys Leu Thr Gly Lys Lys Leu Trp Ser
260 265 270

Ser Glu Asp Phe Ser Thr Leu Asn Ser Asp Met Gly Ala Gly Cys Trp
275 280 285

Gly Arg Ile Leu Asn Gln Asn Tyr Ile Asn Gly Tyr Met Thr Ser Thr
290 295 300

Ile Ala Trp Asn Leu Val Ala Ser Tyr Tyr Glu Gln Leu Pro Tyr Gly
305 310 315 320

Arg Cys Gly Leu Met Thr Ala Gln Glu Pro Trp Ser Gly His Tyr Val
325 330 335

Val Glu Ser Pro Val Trp Val Ser Ala His Thr Thr Gln Phe Thr Gln
340 345 350

Pro Gly Trp Tyr Tyr Leu Lys Thr Val Gly His Leu Glu Lys Gly Gly
355 360 365

Ser Tyr Val Ala Leu Thr Asp Gly Leu Gly Asn Leu Thr Ile Ile Ile
370 375 380

Glu Thr Met Ser His Lys His Ser Lys Cys Ile Arg Pro Phe Leu Pro
385 390 395 400

Tyr Phe Asn Val Ser Gln Gln Phe Ala Thr Phe Val Leu Lys Gly Ser
405 410 415

Phe Ser Glu Ile Pro Glu Leu Gln Val Trp Tyr Thr Lys Leu Gly Lys
420 425 430

Thr Ser Glu Arg Phe Leu Phe Lys Gln Leu Asp Ser Leu Trp Leu Leu
435 440 445

Asp Ser Asp Gly Ser Phe Thr Leu Ser Leu His Glu Asp Glu Leu Phe
450 455 460

Thr Leu Thr Thr Leu Thr Thr Gly Arg Lys Gly Ser Tyr Pro Leu Pro
465 470 475 480

Pro Lys Ser Gln Pro Phe Pro Ser Thr Tyr Lys Asp Asp Phe Asn Val
485 490 495

Asp Tyr Pro Phe Phe Ser Glu Ala Pro Asn Phe Ala Asp Gln Thr Gly
500 505 510

Val Phe Glu Tyr Phe Thr Asn Ile Glu Asp Pro Gly Glu His His Phe
515 520 525

Thr Leu Arg Gln Val Leu Asn Gln Arg Pro Ile Thr Trp Ala Ala Asp
530 535 540

Ala Ser Asn Thr Ile Ser Ile Ile Gly Asp Tyr Asn Trp Thr Asn Leu
545 550 555 560

Thr Ile Lys Cys Asp Val Tyr Ile Glu Thr Pro Asp Thr Gly Gly Val
565 570 575

Phe Ile Ala Gly Arg Val Asn Lys Gly Gly Ile Leu Ile Arg Ser Ala
580 585 590

Arg Gly Ile Phe Phe Trp Ile Phe Ala Asn Gly Ser Tyr Arg Val Thr
595 600 605

Gly Asp Leu Ala Gly Trp Ile Ile Tyr Ala Leu Gly Arg Val Glu Val
610 615 620

Thr Ala Lys Lys Trp Tyr Thr Leu Thr Leu Thr Ile Lys Gly His Phe
625 630 635 640

Thr Ser Gly Met Leu Asn Asp Lys Ser Leu Trp Thr Asp Ile Pro Val
645 650 655

Asn Phe Pro Lys Asn Gly Trp Ala Ala Ile Gly Thr His Ser Phe Glu
660 665 670

Phe Ala Gln Phe Asp Asn Phe Leu Val Glu Ala Thr Arg
675 680 685

<210> 34
<211> 2055

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic polynucleotide

<400> 34

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ggcgcctacg tgctggacga cagcgacggc ctggggccgc agttcgacgg catcggcgcc	180
gtgagcggcg gcggcgccac cagccgcctg ctggtgaact accccgagcc ctaccgcagc	240
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gagatcggcg gcgacggcca gaccaccgac ggcaccgagc ccagccacat gcactacgcc	360
ctggacgaga actacttccg cggctacgag tggtagctga tgaaggaggc caagaagcgc	420
aacccaaca tcacctgat cggcctgccc tggagcttcc ccggctggct gggcaagggc	480
ttcgactggc cctacgtgaa cctgcagctg accgcctact acgtggtgac ctggatcgtg	540
ggcgccaagc gctaccacga cctggacatc gactacatcg gcatctggaa cgagcgcagc	600
tacaacgcca actacatcaa gatcctgcgc aagatgctga actaccaggg cctgcagcgc	660
gtgaagatca tcgccagcga caacctgtgg gagagcatca gcgccagcat gctgctggac	720
gccgagctgt tcaaggtggt ggacgtgatc ggcgcccact accccggcac ccacagcgcc	780
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atgaccagca ccatcgcctg gaacctggtg gccagctact acgagcagct gccctacggc	960
cgctgcggcc tgatgaccgc ccaggagccc tggagcggcc actacgtggt ggagagcccc	1020
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gtgggcccacc tggagaaggg cggcagctac gtggccctga ccgacggcct gggcaacctg	1140
accatcatca tcgagacat gagccacaag cacagcaagt gcatccgccc cttcctgccc	1200
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P109470003W000-SEQ-KZM.txt

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gacgagctgt tcaccctgac caccctgacc accggccgca agggcagcta cccctgccc    1440
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accatcaagt gcgacgtgta catcgagacc ccgacaccg gcggcgtgtt catcgccggc    1740
cgcgtgaaca agggcggcat cctgatccgc agcgcccgcg gcatcttctt ctggatcttc    1800
gccaacggca gctaccgctg gaccggcgac ctggccggct ggatcatcta cgccctgggc    1860
cgcgtggagg tgaccgcaa gaagtggtag accctgacct tgaccatcaa gggccacttc    1920
accagcggca tgctgaacga caagagcctg tggaccgaca tccccgtgaa cttccccaag    1980
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gtggaggcca cccgc                                           2055

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<210> 35
 <211> 339
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic polypeptide

<400> 35

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Ala	Arg	Ser	Arg	Pro	Ser	Phe	His	Pro	Leu	Ser	Asp	Glu	Leu	Val	Asn
			20					25					30		

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Tyr Val Asn Lys Arg Asn Thr Thr Trp Gln Ala Gly His Asn Phe Tyr
35 40 45

Asn Val Asp Met Ser Tyr Leu Lys Arg Leu Cys Gly Thr Phe Leu Gly
50 55 60

Gly Pro Lys Pro Pro Gln Arg Val Met Phe Thr Glu Asp Leu Lys Leu
65 70 75 80

Pro Ala Ser Phe Asp Ala Arg Glu Gln Trp Pro Gln Cys Pro Thr Ile
85 90 95

Lys Glu Ile Arg Asp Gln Gly Ser Cys Gly Ser Cys Trp Ala Phe Gly
100 105 110

Ala Val Glu Ala Ile Ser Asp Arg Ile Cys Ile His Thr Asn Ala His
115 120 125

Val Ser Val Glu Val Ser Ala Glu Asp Leu Leu Thr Cys Cys Gly Ser
130 135 140

Met Cys Gly Asp Gly Cys Asn Gly Gly Tyr Pro Ala Glu Ala Trp Asn
145 150 155 160

Phe Trp Thr Arg Lys Gly Leu Val Ser Gly Gly Leu Tyr Glu Ser His
165 170 175

Val Gly Cys Arg Pro Tyr Ser Ile Pro Pro Cys Glu His His Val Asn
180 185 190

Gly Ser Arg Pro Pro Cys Thr Gly Glu Gly Asp Thr Pro Lys Cys Ser
195 200 205

Lys Ile Cys Glu Pro Gly Tyr Ser Pro Thr Tyr Lys Gln Asp Lys His
210 215 220

P109470003W000-SEQ-KZM.txt

Tyr Gly Tyr Asn Ser Tyr Ser Val Ser Asn Ser Glu Lys Asp Ile Met
225 230 235 240

Ala Glu Ile Tyr Lys Asn Gly Pro Val Glu Gly Ala Phe Ser Val Tyr
245 250 255

Ser Asp Phe Leu Leu Tyr Lys Ser Gly Val Tyr Gln His Val Thr Gly
260 265 270

Glu Met Met Gly Gly His Ala Ile Arg Ile Leu Gly Trp Gly Val Glu
275 280 285

Asn Gly Thr Pro Tyr Trp Leu Val Ala Asn Ser Trp Asn Thr Asp Trp
290 295 300

Gly Asp Asn Gly Phe Phe Lys Ile Leu Arg Gly Gln Asp His Cys Gly
305 310 315 320

Ile Glu Ser Glu Val Val Ala Gly Ile Pro Arg Thr Asp Gln Tyr Trp
325 330 335

Glu Lys Ile

<210> 36

<211> 1017

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic polynucleotide

<400> 36

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tggcaggccg gccacaactt ctacaacgtg gacatgagct acctgaagcg cctgtgcggc 180

accttcctgg gcggcccca gccccccag cgcgtgatgt tcaccgagga cctgaagctg 240

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cccgccagct tcgacgcccg cgagcagtgg cccagtgcc ccaccatcaa ggagatccgc      300
gaccagggca gctgcggcag ctgctgggcc ttcggcgccg tggaggccat cagcgaccgc      360
atctgcatcc acaccaacgc ccacgtgagc gtggaggtga gcgccgagga cctgctgacc      420
tgctgcggca gcatgtgcgg cgacggctgc aacggcggct accccgccga ggcctggaac      480
ttctggaccc gcaagggcct ggtgagcggc ggcctgtacg agagccacgt gggctgccgc      540
ccctacagca tccccccctg cgagcaccac gtgaacggca gccgcccccc ctgcaccggc      600
gagggcgaca cccccaagtg cagcaagatc tgcgagcccg gctacagccc cacctacaag      660
caggacaagc actacggcta caacagctac agcgtgagca acagcgagaa ggacatcatg      720
gccgagatct acaagaacgg ccccggtggag ggcgccctta gcgtgtacag cgacttcctg      780
ctgtacaaga gcggcgtgta ccagcacgtg accggcgaga tgatgggcgg ccacgccatc      840
cgcatcctgg gctggggcgt ggagaacggc accccctact ggctggtggc caacagctgg      900
aacaccgact ggggcgacaa cggcttcttc aagatcctgc gcggccagga cactgcggc      960
atcgagagcg aggtggtggc cggcatcccc cgcaccgacc agtactggga gaagatc     1017

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<210> 37
 <211> 631
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic polypeptide

<400> 37

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Met Pro Arg Tyr Gly Ala Ser Leu Arg Gln Ser Cys Pro Arg Ser Gly
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Arg Glu Gln Gly Gln Asp Gly Thr Ala Gly Ala Pro Gly Leu Leu Trp
          20          25          30

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Met Gly Leu Val Leu Ala Leu Ala Leu Ala Leu Ala Leu Ala
35          40          45

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Leu Ser Asp Ser Arg Val Leu Trp Ala Pro Ala Glu Ala His Pro Leu
50 55 60

Ser Pro Gln Gly His Pro Ala Arg Leu His Arg Ile Val Pro Arg Leu
65 70 75 80

Arg Asp Val Phe Gly Trp Gly Asn Leu Thr Cys Pro Ile Cys Lys Gly
85 90 95

Leu Phe Thr Ala Ile Asn Leu Gly Leu Lys Lys Glu Pro Asn Val Ala
100 105 110

Arg Val Gly Ser Val Ala Ile Lys Leu Cys Asn Leu Leu Lys Ile Ala
115 120 125

Pro Pro Ala Val Cys Gln Ser Ile Val His Leu Phe Glu Asp Asp Met
130 135 140

Val Glu Val Trp Arg Arg Ser Val Leu Ser Pro Ser Glu Ala Cys Gly
145 150 155 160

Leu Leu Leu Gly Ser Thr Cys Gly His Trp Asp Ile Phe Ser Ser Trp
165 170 175

Asn Ile Ser Leu Pro Thr Val Pro Lys Pro Pro Pro Lys Pro Pro Ser
180 185 190

Pro Pro Ala Pro Gly Ala Pro Val Ser Arg Ile Leu Phe Leu Thr Asp
195 200 205

Leu His Trp Asp His Asp Tyr Leu Glu Gly Thr Asp Pro Asp Cys Ala
210 215 220

Asp Pro Leu Cys Cys Arg Arg Gly Ser Gly Leu Pro Pro Ala Ser Arg
225 230 235 240

Pro Gly Ala Gly Tyr Trp Gly Glu Tyr Ser Lys Cys Asp Leu Pro Leu
245 250 255

Arg Thr Leu Glu Ser Leu Leu Ser Gly Leu Gly Pro Ala Gly Pro Phe
260 265 270

Asp Met Val Tyr Trp Thr Gly Asp Ile Pro Ala His Asp Val Trp His
275 280 285

Gln Thr Arg Gln Asp Gln Leu Arg Ala Leu Thr Thr Val Thr Ala Leu
290 295 300

Val Arg Lys Phe Leu Gly Pro Val Pro Val Tyr Pro Ala Val Gly Asn
305 310 315 320

His Glu Ser Thr Pro Val Asn Ser Phe Pro Pro Pro Phe Ile Glu Gly
325 330 335

Asn His Ser Ser Arg Trp Leu Tyr Glu Ala Met Ala Lys Ala Trp Glu
340 345 350

Pro Trp Leu Pro Ala Glu Ala Leu Arg Thr Leu Arg Ile Gly Gly Phe
355 360 365

Tyr Ala Leu Ser Pro Tyr Pro Gly Leu Arg Leu Ile Ser Leu Asn Met
370 375 380

Asn Phe Cys Ser Arg Glu Asn Phe Trp Leu Leu Ile Asn Ser Thr Asp
385 390 395 400

Pro Ala Gly Gln Leu Gln Trp Leu Val Gly Glu Leu Gln Ala Ala Glu
405 410 415

Asp Arg Gly Asp Lys Val His Ile Ile Gly His Ile Pro Pro Gly His
420 425 430

Cys Leu Lys Ser Trp Ser Trp Asn Tyr Tyr Arg Ile Val Ala Arg Tyr
435 440 445

Glu Asn Thr Leu Ala Ala Gln Phe Phe Gly His Thr His Val Asp Glu
450 455 460

Phe Glu Val Phe Tyr Asp Glu Glu Thr Leu Ser Arg Pro Leu Ala Val
465 470 475 480

Ala Phe Leu Ala Pro Ser Ala Thr Thr Tyr Ile Gly Leu Asn Pro Gly
485 490 495

Tyr Arg Val Tyr Gln Ile Asp Gly Asn Tyr Ser Gly Ser Ser His Val
500 505 510

Val Leu Asp His Glu Thr Tyr Ile Leu Asn Leu Thr Gln Ala Asn Ile
515 520 525

Pro Gly Ala Ile Pro His Trp Gln Leu Leu Tyr Arg Ala Arg Glu Thr
530 535 540

Tyr Gly Leu Pro Asn Thr Leu Pro Thr Ala Trp His Asn Leu Val Tyr
545 550 555 560

Arg Met Arg Gly Asp Met Gln Leu Phe Gln Thr Phe Trp Phe Leu Tyr
565 570 575

His Lys Gly His Pro Pro Ser Glu Pro Cys Gly Thr Pro Cys Arg Leu
580 585 590

Ala Thr Leu Cys Ala Gln Leu Ser Ala Arg Ala Asp Ser Pro Ala Leu
595 600 605

Cys Arg His Leu Met Pro Asp Gly Ser Leu Pro Glu Ala Gln Ser Leu
610 615 620

Trp Pro Arg Pro Leu Phe Cys
625 630

<210> 38
<211> 1896
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic polynucleotide

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gaggacgaca tgggtggaggt gtggcgccgc agcgtgctga gcccagcga ggcctgcggc 480
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cccaccgtgc ccaagcccc cccaagccc ccagcccc ccgcccccg cgccccctg 600
agccgcatcc tgttcctgac cgacctgcac tgggaccacg actacctgga gggcaccgac 660
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cccggcgccg gctactgggg cgagtacagc aagtgcgacc tgcccctgcg caccctggag 780
agcctgctga gcggcctggg ccccgccggc cccttcgaca tgggtgtactg gaccggcgac 840
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cacgagagca ccccggtgaa cagcttcccc ccccccttca tcgagggcaa ccacagcagc 1020

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aaggtgcaca tcatcggcca catccccccc ggccactgcc tgaagagctg gagctggaac      1320
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cacgtggacg agttcgaggt gttctacgac gaggagaccc tgagccgccc cctggccgtg      1440
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cagatcgacg gcaactacag cggcagcagc cacgtggtgc tggaccacga gacctacatc      1560
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gcccgcgccc acagccccgc cctgtgccgc cacctgatgc ccgacggcag cctgcccag      1860
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<210> 39
<211> 11329
<212> DNA
<213> Artificial sequence

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<220>
<223> Synthetic polynucleotide

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<400> 39
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gccaactcca tcactagggg ttcttgctag ctctgggtat ttaagcccga gtgagcacgc      180
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agccgagaac gaaccttctg ctggactgct gagcggctac ccctttcagt gcctgggctt	1500
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caccacccat aatgtgcggc tgctgatgct ggacgaccag agactgcttc tgccccactg	1620
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cgtgcggctc ggagctggg atagaggcat gcagtacagc cacagcatca tcaccaacct	1860
gctgtaccac gtcgtcggct ggaccgactg gaatctggcc ctgaatcctg aaggcggccc	1920
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ccctgatgga tctgctgtgg tgggtggtcct gaaccgcagc agcaaagatg tgcccctgac	2160
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ctacctgtgg cgtagacagg agggcagagg aagtcttctg acatgcggag acgtggaaga	2280
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 <400> 45

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Arg Cys Ser Asn Gly Phe Pro Glu Arg Asp Pro Pro Arg Pro Gly Pro
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Ser Arg Pro Ala Glu Lys Pro Pro Arg Pro Glu Ala Lys Ser Ala Gln
35 40 45

Pro Ala Asp Gly Trp Lys Gly Glu Arg Pro Arg Ser Glu Glu Asp Asn
50 55 60

Glu Leu Asn Leu Pro Asn Leu Ala Ala Ala Tyr Ser Ser Ile Leu Ser
65 70 75 80

Ser Leu Gly Glu Asn Pro Gln Arg Gln Gly Leu Leu Lys Thr Pro Trp
85 90 95

Arg Ala Ala Ser Ala Met Gln Phe Phe Thr Lys Gly Tyr Gln Glu Thr
100 105 110

Ile Ser Asp Val Leu Asn Asp Ala Ile Phe Asp Glu Asp His Asp Glu
115 120 125

Met Val Ile Val Lys Asp Ile Asp Met Phe Ser Met Cys Glu His His
130 135 140

Leu Val Pro Phe Val Gly Lys Val His Ile Gly Tyr Leu Pro Asn Lys
145 150 155 160

Gln Val Leu Gly Leu Ser Lys Leu Ala Arg Ile Val Glu Ile Tyr Ser
165 170 175

Arg Arg Leu Gln Val Gln Glu Arg Leu Thr Lys Gln Ile Ala Val Ala
180 185 190

P109470003W000-SEQ-KZM.txt

Ile Thr Glu Ala Leu Arg Pro Ala Gly Val Gly Val Val Val Glu Ala
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Thr His Met Cys Met Val Met Arg Gly Val Gln Lys Met Asn Ser Lys
210 215 220

Thr Val Thr Ser Thr Met Leu Gly Val Phe Arg Glu Asp Pro Lys Thr
225 230 235 240

Arg Glu Glu Phe Leu Thr Leu Ile Arg Ser
245 250

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ggcgtggggcg tgggtggtgga ggccaccac atgtgcatgg tgatgcgcgg cgtgcagaag 660
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cgcgaggagt tcctgaccct gatccgcagc

750

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<211> 203

<212> PRT

<213> Artificial sequence

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<223> Synthetic polypeptide

<400> 47

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 20 25 30

Ser Lys His Tyr Lys Ser Thr Val Gly Val Asp Phe Ala Leu Lys Val
 35 40 45

Leu Gln Trp Ser Asp Tyr Glu Ile Val Arg Leu Gln Leu Trp Asp Ile
 50 55 60

Ala Gly Gln Glu Arg Phe Thr Ser Met Thr Arg Leu Tyr Tyr Arg Asp
 65 70 75 80

Ala Ser Ala Cys Val Ile Met Phe Asp Val Thr Asn Ala Thr Thr Phe
 85 90 95

Ser Asn Ser Gln Arg Trp Lys Gln Asp Leu Asp Ser Lys Leu Thr Leu
 100 105 110

Pro Asn Gly Glu Pro Val Pro Cys Leu Leu Leu Ala Asn Lys Cys Asp
 115 120 125

Leu Ser Pro Trp Ala Val Ser Arg Asp Gln Ile Asp Arg Phe Ser Lys
 130 135 140

Glu Asn Gly Phe Thr Gly Trp Thr Glu Thr Ser Val Lys Glu Asn Lys
145 150 155 160

Asn Ile Asn Glu Ala Met Arg Val Leu Ile Glu Lys Met Met Arg Asn
165 170 175

Ser Thr Glu Asp Ile Met Ser Leu Ser Thr Gln Gly Asp Tyr Ile Asn
180 185 190

Leu Gln Thr Lys Ser Ser Ser Trp Ser Cys Cys
195 200

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<211> 609
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic polynucleotide

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aacatcaacg aggccatgcg cgtgctgatc gagaagatga tgcgcaacag caccgaggac 540
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agctgctgc 609

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 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic polypeptide

<400> 49

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Asp Glu Ala Ile Gln Ala Val Lys Val Gln Ser Phe Gln Met Lys Arg
 20 25 30

Cys Leu Asp Lys Asn Lys Leu Met Asp Ala Leu Lys His Ala Ser Asn
 35 40 45

Met Leu Gly Glu Leu Arg Thr Ser Met Leu Ser Pro Lys Ser Tyr Tyr
 50 55 60

Glu Leu Tyr Met Ala Ile Ser Asp Glu Leu His Tyr Leu Glu Val Tyr
 65 70 75 80

Leu Thr Asp Glu Phe Ala Lys Gly Arg Lys Val Ala Asp Leu Tyr Glu
 85 90 95

Leu Val Gln Tyr Ala Gly Asn Ile Ile Pro Arg Leu Tyr Leu Leu Ile
 100 105 110

Thr Val Gly Val Val Tyr Val Lys Ser Phe Pro Gln Ser Arg Lys Asp
 115 120 125

Ile Leu Lys Asp Leu Val Glu Met Cys Arg Gly Val Gln His Pro Leu
 130 135 140

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Arg Gly Leu Phe Leu Arg Asn Tyr Leu Leu Gln Cys Thr Arg Asn Ile
145 150 155 160

Leu Pro Asp Glu Gly Glu Pro Thr Asp Glu Glu Thr Thr Gly Asp Ile
165 170 175

Ser Asp Ser Met Asp Phe Val Leu Leu Asn Phe Ala Glu Met Asn Lys
180 185 190

Leu Trp Val Arg Met Gln His Gln Gly His Ser Arg Asp Arg Glu Lys
195 200 205

Arg Glu Arg Glu Arg Gln Glu Leu Arg Ile Leu Val Gly Thr Asn Leu
210 215 220

Val Arg Leu Ser Gln Leu Glu Gly Val Asn Val Glu Arg Tyr Lys Gln
225 230 235 240

Ile Val Leu Thr Gly Ile Leu Glu Gln Val Val Asn Cys Arg Asp Ala
245 250 255

Leu Ala Gln Glu Tyr Leu Met Glu Cys Ile Ile Gln Val Phe Pro Asp
260 265 270

Glu Phe His Leu Gln Thr Leu Asn Pro Phe Leu Arg Ala Cys Ala Glu
275 280 285

Leu His Gln Asn Val Asn Val Lys Asn Ile Ile Ile Ala Leu Ile Asp
290 295 300

Arg Leu Ala Leu Phe Ala His Arg Glu Asp Gly Pro Gly Ile Pro Ala
305 310 315 320

Asp Ile Lys Leu Phe Asp Ile Phe Ser Gln Gln Val Ala Thr Val Ile
325 330 335

P109470003W000-SEQ-KZM.txt

Gln Ser Arg Gln Asp Met Pro Ser Glu Asp Val Val Ser Leu Gln Val
340 345 350

Ser Leu Ile Asn Leu Ala Met Lys Cys Tyr Pro Asp Arg Val Asp Tyr
355 360 365

Val Asp Lys Val Leu Glu Thr Thr Val Glu Ile Phe Asn Lys Leu Asn
370 375 380

Leu Glu His Ile Ala Thr Ser Ser Ala Val Ser Lys Glu Leu Thr Arg
385 390 395 400

Leu Leu Lys Ile Pro Val Asp Thr Tyr Asn Asn Ile Leu Thr Val Leu
405 410 415

Lys Leu Lys His Phe His Pro Leu Phe Glu Tyr Phe Asp Tyr Glu Ser
420 425 430

Arg Lys Ser Met Ser Cys Tyr Val Leu Ser Asn Val Leu Asp Tyr Asn
435 440 445

Thr Glu Ile Val Ser Gln Asp Gln Val Asp Ser Ile Met Asn Leu Val
450 455 460

Ser Thr Leu Ile Gln Asp Gln Pro Asp Gln Pro Val Glu Asp Pro Asp
465 470 475 480

Pro Glu Asp Phe Ala Asp Glu Gln Ser Leu Val Gly Arg Phe Ile His
485 490 495

Leu Leu Arg Ser Glu Asp Pro Asp Gln Gln Tyr Leu Ile Leu Asn Thr
500 505 510

Ala Arg Lys His Phe Gly Ala Gly Gly Asn Gln Arg Ile Arg Phe Thr
515 520 525

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Leu Pro Pro Leu Val Phe Ala Ala Tyr Gln Leu Ala Phe Arg Tyr Lys
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Glu Asn Ser Lys Val Asp Asp Lys Trp Glu Lys Lys Cys Gln Lys Ile
545 550 555 560

Phe Ser Phe Ala His Gln Thr Ile Ser Ala Leu Ile Lys Ala Glu Leu
565 570 575

Ala Glu Leu Pro Leu Arg Leu Phe Leu Gln Gly Ala Leu Ala Ala Gly
580 585 590

Glu Ile Gly Phe Glu Asn His Glu Thr Val Ala Tyr Glu Phe Met Ser
595 600 605

Gln Ala Phe Ser Leu Tyr Glu Asp Glu Ile Ser Asp Ser Lys Ala Gln
610 615 620

Leu Ala Ala Ile Thr Leu Ile Ile Gly Thr Phe Glu Arg Met Lys Cys
625 630 635 640

Phe Ser Glu Glu Asn His Glu Pro Leu Arg Thr Gln Cys Ala Leu Ala
645 650 655

Ala Ser Lys Leu Leu Lys Lys Pro Asp Gln Gly Arg Ala Val Ser Thr
660 665 670

Cys Ala His Leu Phe Trp Ser Gly Arg Asn Thr Asp Lys Asn Gly Glu
675 680 685

Glu Leu His Gly Gly Lys Arg Val Met Glu Cys Leu Lys Lys Ala Leu
690 695 700

Lys Ile Ala Asn Gln Cys Met Asp Pro Ser Leu Gln Val Gln Leu Phe
705 710 715 720

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Ile Glu Ile Leu Asn Arg Tyr Ile Tyr Phe Tyr Glu Lys Glu Asn Asp
725 730 735

Ala Val Thr Ile Gln Val Leu Asn Gln Leu Ile Gln Lys Ile Arg Glu
740 745 750

Asp Leu Pro Asn Leu Glu Ser Ser Glu Glu Thr Glu Gln Ile Asn Lys
755 760 765

His Phe His Asn Thr Leu Glu His Leu Arg Leu Arg Arg Glu Ser Pro
770 775 780

Glu Ser Glu Gly Pro Ile Tyr Glu Gly Leu Ile Leu
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<212> DNA
<213> Artificial sequence

<220>
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Glu Glu Cys Thr Val Thr Gly Phe Leu Arg Asp Lys Leu Gln Tyr Arg
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Ser Arg Leu Gln Tyr Met Lys His Tyr Phe Pro Ile Asn Tyr Lys Ile
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Ser Val Pro Tyr Glu Gly Val Phe Arg Ile Ala Asn Val Thr Arg Leu
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Gln Arg Ala Gln Val Ser Glu Arg Glu Leu Arg Tyr Leu Trp Val Leu
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Val Ser Leu Ser Ala Thr Glu Ser Val Gln Asp Val Leu Leu Glu Gly
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His Pro Ser Trp Lys Tyr Leu Gln Glu Val Glu Thr Leu Leu Leu Asn
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Val Gln Gln Gly Leu Thr Asp Val Glu Val Ser Pro Lys Val Glu Ser
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Pro Lys Ala Leu Leu Asp Asn Cys Phe Arg Val Met Glu Leu Leu Tyr
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Cys Ser Cys Cys Lys Gln Ser Ser Val Leu Asn Trp Gln Asp Cys Glu
 180 185 190

Val Pro Ser Pro Gln Ser Cys Ser Pro Glu Pro Ser Leu Gln Tyr Ala
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Ser Thr His Asn Leu Trp Leu Leu Ser Phe Leu Arg Arg Trp Asn Gly
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Ser Thr Ala Ile Thr Asp Asp Thr Leu Gly Gly Thr Leu Thr Ile Thr
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Leu His Gly Ser Glu Ala Asp Thr Leu Arg Lys Val Leu Val Glu Val
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